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Contamination trends of legacy and emerging compounds in sediments from the Adriatic Sea

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Abstract

Coastal areas are under continuous and increasing pressure from different human activities. A cocktail of contaminants (e.g. hydrocarbons, pesticides, persistent organic pollutants (POPs), emerging contaminants, and others) threatens water, sediment and biota. Estimates suggest that more than 100,000 chemicals are currently in use for different purposes around the world and the majority of these substances are discharged into waterbodies, reaching coastal and marine systems. Sediments are the final repositories for most pollutants, which can accumulate and remain in the sedimentary matrix for long periods of time and may accumulate through the food web, affecting marine biota, aquatic-dependent wildlife, and ultimately human health. Thus, a wide-ranging work monitoring different groups of contaminants in sediments is a key tool for a comprehensive understanding of contaminants behavior and identification of the overall environmental quality and possible threatens to the whole marine ecosystem. This thesis contributes to a better understanding on the occurrence, levels, distribution, historical use, potential sources and environmental behaviour of selected groups of legacy and emerging compounds in sediments from the Adriatic Sea. POPs, among which PCBs and DDTs rank first, polycyclic aromatic hyrocarbons (PAHs), and selected groups contaminants of emerging concern (CECs) (fragrances, UV filters, endocrine disruptors) were the objective of this work. First, PCB congeners were analyzed in sediment cores and recent sediments along the north-south axis of the western Adriatic Sea. Chronological records of PCB concentrations and homologue patterns were assessed, supporting the reconstruction of PCBs fluxes and total inventories. Then, the spatial distribution and fate of selected legacy and emerging compounds were investigated in surface sediments sampled along the Adriatic mud-wedge and in deep-sea regions from the southern Adriatic basin, providing the first extensive dataset and also the evaluation of potential ecotoxicological risk of target emerging contaminants in sediments from the Adriatic Sea. Finally, the analyzes of selected legacy contaminants (PCBs, DDTs and PAHs) in sediment cores from coastal and deep-sea sediments from the Adriatic Sea supported the comparison between these two areas and further reinforces the hypothesis on the transfer of contaminants from contaminated areas in the northern Adriatic to the deep southern Adriatic basin. The present thesis is the summary of the work carried out in the last three year of the PhD

program “Scienze ambientali: tutela e gestione delle risorse naturali” and contains three manuscripts (two under review and one in preparation), and the main conclusions regarding this PhD research project.

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"*No man is an island*"

(John Donne)

Contents

Chapter 1

Introduction, study area and objectives

1.1 Introduction

Marine and coastal areas are facing continuous and increasing impacts arising out of human activities. Threats to marine ecosystems include climate change, aquaculture, fishing, species invasion and pollutant inputs causing effects such as physical and chemical transformation, habitat destruction and changes in biodiversity (Borja et al., 2008; Halpern et al., 2007). Understanding the extension and consequences of human impacts is needed for the evaluation of sustainable uses of oceans and protection of marine ecosystems at all scales (Halpern, 2008).

Marine and coastal areas are the final receptacle for human waste and contaminants, whether it is by deliberate dumping or by natural runoff from the land (Sahu et al., 2009). Contamination sources are mainly located in populated, industrialized and agricultural areas, where anthropogenic contaminants can reach the marine environment by wastewater discharge, soil runoffs, sewage outfalls, leaching from agriculture, direct spillages into soils, and volatilization (Breivik et al., 2002a; Litskas et al., 2012; Zhou et al., 2001). Contaminants are transported from the sources areas especially through atmospheric deposition and water transport, reaching residential and background areas, where sediments usually act as a sink for most pollutants (Argiriadis et al., 2014; Sahu et al., 2009).

Depending on specific physical-chemical characteristics (e.g. octanol-water partition coefficient and chemical stability), some classes of contaminants can accumulate and persist in the sedimentary matrix for long periods of time, from years to several decades (Ruiz-Fernández et al., 2012). As a consequence, sediments can represent a record of contamination levels, representing a helpful instrument to understand the overall environmental quality of marine systems. For instance, the analysis of surface sediments can be used to assess the spatial distribution of chemicals, while sediment

cores can represent the historical inputs and trends of contaminants (Hong et al., 2003; Martins et al., 2010; Santschi et al., 2001).

Sediments can also serve as secondary source of contaminants because they can be subject to remobilization, transport, and redistribution during certain environmental (e.g. bioturbation, chemical processes and flood events) or human activities (e.g. dredging and relocation of sediments) (Holoubek and Klánová, 2008). Thus, the presence of contaminants in the sedimentary matrix is also a threat to the general environmental health, since several chemicals have been proven to be potentially toxic and can accumulate through the food web, affecting surrounding biota, aquatic-dependent organisms, and human health (Borgå et al., 2001; Vallack et al., 1998).

There has been a growing concern over the last decades about the environmental distribution and the potential effects of synthetic substances. Environmental policies and efforts are being made at different levels (from local to international level) in order to achieve a comprehensive understanding and protection of marine systems. For example, the Marine Strategy Framework Directive (MSFD) is the legal instrument that has been adopted by the European Union (EU) in order to achieve Good Environmental Status (GES) of the EU's marine waters by 2020 (European Commission, 2008). The MSFD is the first of EU directives that takes a holistic, functional approach and is based on the use of an integrated analysis of the marine ecosystem, identifying a set of 11 descriptors of GES that aims to represent, protect and understand the functioning of the system as a whole (Borja et al., 2008; Crise et al., 2015).

The set of 11 descriptors consist of a wide range of environmental indicators, including maintenance of biological diversity, introduction of alien species, fishing and eutrophication. Among these, descriptor 8 ("Concentrations of contaminants are at levels not giving rise to pollution effects") addresses data availability, knowledge gaps and research priorities regarding contaminants and marine pollution impacts (Figure 1).

According to the European legislation contaminants are "substances (i.e. chemical elements and compounds) or groups of substances that are toxic, persistent and liable to bio-accumulate and other substances or groups of substances which give rise to an equivalent level of concern" (Directive 2000/60/EC). To this end, the Environmental Quality Standard Directive (Directive 2008/105/EC) establishes requirements for the chemical status of surface waters including marine waters defining an Environmental Quality Standard (EQS), which is the maximum allowable concentration of a

contaminant not causing harm. Environmental Quality Standards for "Priority Substances and certain other pollutants" can be find in Annex I to the EQS Directive.

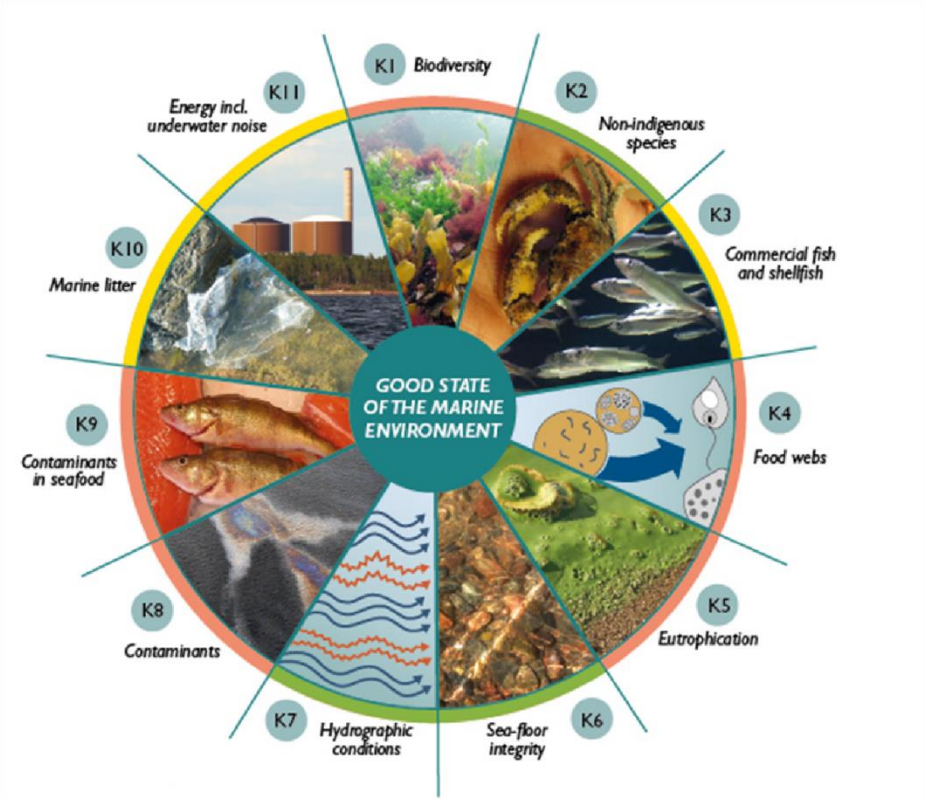

Figure 1. Qualitative descriptors describing environmental status under MSFD. Adapted from "State of the Baltic Sea" (http://www.ymparisto.fi/en-US/Sea/Baltic_Sea_ecosystem_wheel_presents_elev(35474)

In recent decades, numerous studies have been published regarding contaminant concentrations and marine pollution (e.g. Hong et al., 1999; Lara-Martín et al., 2010; Notar et al., 2001; Tolosa et al., 1995; Wang et al., 2015). However, despite the availability of an important amount of data sets and long time series for several contaminants families, there is still lack of data for specific contaminants, especially regarding contaminants of emerging concern (CEC; e.g. fragrances and UV filters). For legacy compounds (e.g. polychlorinated biphenyls - PCBs, polycyclic aromatic hydrocarbons – PAHs, and chlorinated pesticides), most of the data available refers to restricted areas and closed or semi-enclosed seas, and a lack of off-shore datasets has been detected (Crise et al., 2015).

The Mediterranean Sea has been recognized as a marine region of special concern from the environmental point of view due to its particular hydrographical characteristics. It is a semi-enclosed basin with a negative water budget, where evaporation exceeds Atlantic Ocean through the Strait of Gibraltar. This feature has significant implications for the accumulation of POPs from point and diffuse land based sources (UNEP/MAP, 2001; Gómez-Gutiérrez et al., 2007). The aim of PERSEUS is the gathering of information about sources, environmental concentrations and effects of pollutants in the region. However, despite the efforts done during the last 30 years, there is still a scarcity of data for many legacy and emerging contaminants and geographical areas, and the information is not always adequately assessed in order to be useful to assess the state of marine pollution according to the objectives of the MSFD.

1.1.1 Legacy contaminants

Legacy contaminants refers to well-known groups of contaminants that have been monitored and regulated in most parts of the world, being also referred to as "regulated contaminants". Persistent organic pollutants (POPs) are among the most concerning groups of legacy contaminants due to their specific characteristics as high persistence in the environment, hydrophobicity, toxicity and related adverse effects, including endocrine disrupting and carcinogenic effects and capacity of biomagnification (Borgå et al., 2001; Cipro et al., 2012; Jones and de Voogt, 1999). Due to their long-range atmospheric transport potential and harmful effects on man and wildlife, regulatory efforts and international agreements have been made in past decades in order to reduce future environmental burdens (Breivik et al., 2004; Vallack et al., 1998; Scheringer et al., 2004).

POPs are semi-volatile chemicals, and after their release into the environment, they travel in multiple cycles of evaporation, transport by air and condensation. Temperature plays an important role in controlling transport and sinks at the global scale through the processes of cold condensation, global distillation, and latitudinal fractionation (Scheringer et al., 2004; Semeena and Lammel, 2005; Jurado and Datchs, 2008). In the cold climate of the Arctic, low evaporation rates trap POPs, and so they enter the food chain (Figure 2). Conversely, deposition is in the mid-high latitudes is driven by sinking

marine particulate matter, rather than by a cold condensation effect (Gustafsson et al., 1997).

Ocean biogeochemical processes play a critical role in controlling the global dynamics and the ultimate sink of POPs (Wania and Mackay, 1996). Even though POP concentrations in the open ocean are lower than those observed in coastal areas, the large oceanic volumes imply that they may represent an important inventory of POPs (Dachs et al., 2002; Jurado et al., 2004; Ma et al., 2015). This has been confirmed in budgets performed for some marine regions, such as the Western Mediterranean (Datchs et al., 1997), and may be true for the global oceans.

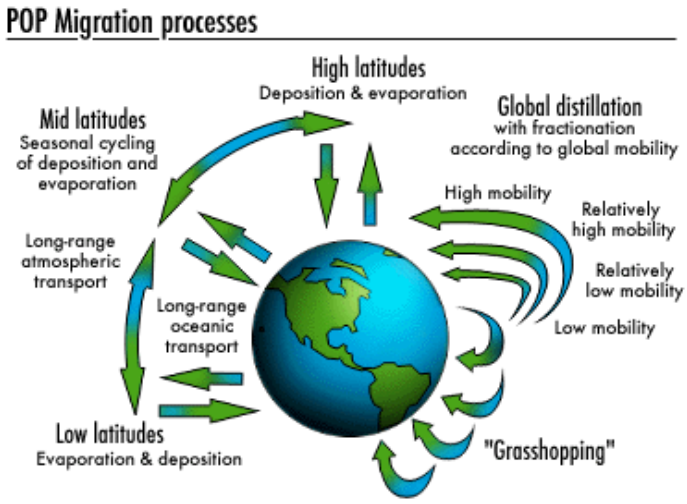

Figure 2. Persistent organic pollutants (POPs) spread via a variety of mechanisms at different latitude (UNEP, 2002).

POPs are internationally regulated within a program of United Nations Environment Program (UNEP) that set the basis for the development of the Stockholm Convention, in 2001. Under the Stockholm Convention, the signatory countries are legally required to eliminate the production, use, and emissions of POPs, with the ultimate goal of reducing human and ecosystem exposure (Holoubek and Klánová, 2008; Nizzetto et al., 2010). Parallel efforts have been made at European level; for instance the recent Directive 2013/39/EU established a list of 45 substances identified for priority action at Union level, including POPs, contaminants of emerging concerns and some trace metals (Viganò et al., 2015).

The regulatory actions have led to the reduction or elimination of major primary sources associated with the production and use of POPs, followed by a gradual decline on environmental levels of POPs over time, especially after the 1980's (Franců et al., 2009; Smith et al., 2009; Zaborska et al., 2011; Combi et al., 2013). However, there are still ongoing primary releases from diffuse sources that are difficult to target for reduction or elimination, such as volatilization from old stockpiles, or from old equipment that is still in use (Breivik et al., 2002a; Nizzetto et al., 2010). Additionally, these compounds are highly persistent in the environment and continue to be found in different environmental matrixes and their current levels are not expected to decrease significantly within the next decades (Breivik et al., 2007; Sobek et al., 2015). Therefore, it is essential to understand the contaminant loadings, both that we may assess the effects of environmental legislation and that we may identify emerging sources of pollution (Kannan et al., 2005).

There are many thousands of POP chemicals, often coming from certain series or "families" of chemicals (Jones and de Voogt, 1999). Among the most important groups of POPs are the chlorinated compounds, including polychlorinated biphenyls (PCBs) and dichlorodiphenyl-trichloroethanes (DDTs), and some groups of polycyclic aromatic hydrocarbons (PAHs).

- *PCBs*

The general molecular formula for the PCBs is $C_{12}H_{10-n}Cl_n$ (Figure 3), where *n* could be any number from 1 to 10 (Breivik et al., 2002b). Theoretically, there are 209 distinct PCB congeners and 10 distinct PCB homologues, dependent on the number of chlorines attached to the rings (Erickson and Kaley, 2011; Wu et al., 2012). The physico-chemical properties of PCBs are dependent on their degree of chlorination; water solubility, vapor pressure and biodegradability decrease with an increasing degree of chlorine substitution while hydrophobicity and sorption tendency increase (Vallack et al., 1998). PCBs present high chemical and thermal resistance, low electric conductivity, high thermal conductivity and present flame retardant properties, making them suitable for a broad range of applications (Erickson and Kaley, 2011; Fiedler, 1997).

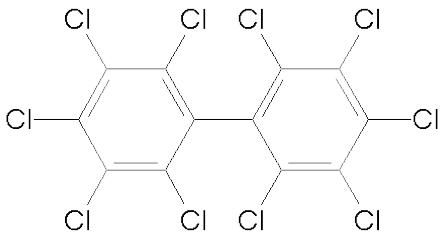

Figure 3. General chemical structure of PCBs.

The PCBs are commonly considered as key representatives of the ‘‘industrial’’ POPs and were massively produced from the 1930s to the beginning of the 1980s in industrialized countries (Breivik et al., 2004; Hosoda et al., 2014). Global production of PCBs had been estimated to exceeded 1.3 million tons and the USA (by Monsanto), Germany (by Bayer AG), France (by Prodelec) and Russia (by Orgsteklo) are among the major producers countries (Breivik et al., 2002a and references therein; Fiedler, 1997). PCB production includes the chlorination of biphenyl in the presence of a catalyst (e.g. ferric chloride) and they were commercialized as technical mixtures with different degrees of chlorination known as Aroclor (Erickson and Kaley, 2011). Foreign manufacturers sold similar products under trade names such as Kanechlor (Japan), Clophen (Germany), Phenoclor and Pyralene (France), Fenchlor (Italy), Sovol (Russia), Chlorfen (Poland), and Delor (the former Czechoslovakia).

Commercial PCB mixtures were used in a wide variety of applications, including electric fluids in transformers and capacitors, hydraulic lubricants, heat transfer fluids, flame retardants, lubricating oils, adhesives, plasticizers, carbonless copy paper, paints, among numerous other applications (Borja et al., 2005; Erickson and Kaley, 2011; Fiedler, 1997). Additionally, PCBs can also be formed to a lesser extension as by-products, or inadvertently generated products, during industrial (e.g. pigments production) and combustion process (Guo et al., 2014; Ishikawa et al., 2007; Rodenburg et al., 2010).

In spite of the production and use ban, PCBs are still in use (in closed systems, e.g., electrical transformers) and broadly distributed in the environment (Hornbuckle and Robertson, 2010). The environmental fate of PCBs is also influenced by their degree of chlorination. Low-chlorinated PCBs (up to 4 chlorine atoms) are more volatile and less

persistent in the marine environment, being usually related to long-range transport and/or dechlorination processes, while the higher chlorinated compounds are associated to local input sources and contaminated areas (Tolosa et al., 1995; Borja et al., 2005).

PCBs have been intensively studied in the last four decades or so. However, there are still current challenges on PCB research, including the uncertainties of how PCBs may interact with other environmental contaminants and the difficulties and costs inherent to PCB analysis (e.g. determination of the complex mixtures of many individual congeners) and the publication rate shows no sign of slowing (Erickson and Kaley, 2011; Hornbuckle and Robertson, 2010).

- *DDTs*

According to the UNEP, pesticides are substances used for controlling disease vectors and agricultural pests because of their toxicity towards target species. Although the pesticide usage dates back to ancient times, the progress in pest control had been minimal until more recent times (Taylor et al., 2007). During the 19th and early 20th century sulfuric acid, iron sulfate, copper sulfate, mercury-containing pesticides and lead arsenate were used on agriculture, especially in Europe and in the USA (Hughes et al., 2011; Taylor et al., 2007). Modern pesticides, including chlorinated hydrocarbon pesticides, started to be produced in the late 1930's – early 1940's.

The insecticidal potential of DDT was first reported in 1939 and it was first used to for typhus and malaria control in wartime (Smith, 2010). Large-scale industrial production and commercial sales started in 1943 for the control of agricultural and forest pests (Turusov et al., 2002). The structure of *p,p′*-DDT and the structures of its degradation products DDD (dichloro-diphenyl-ethane) and DDE (dichloro-diphenyl-ethylene) are showed in Figure 4.

DDD (*o,p′*-DDD and *p,p′*-DDD) and DDE (*o,p′*-DDE and *p,p′*-DDE) are metabolites of DDT (*o,p′*-DDT and *p,p′*-DDT) under anaerobic and aerobic conditions, respectively (Peng et al., 2005; Yu et al., 2011). Although *p,p′*-DDD retains reasonable levels of toxicity for some insect, the main insecticidal active ingredient of technical DDT is *p,p′*-DDT (Matsumura, 2009). Technical-grade DDT contains 65–80% *p,p′*-DDT, 15–21% *o,p′*-DDT, up to 14% *p,p′*- and *o,p′*-DDD, and around 4% of *p,p′*- and *o,p′*-DDE (Matsumura, 2009; Smith, 2010).

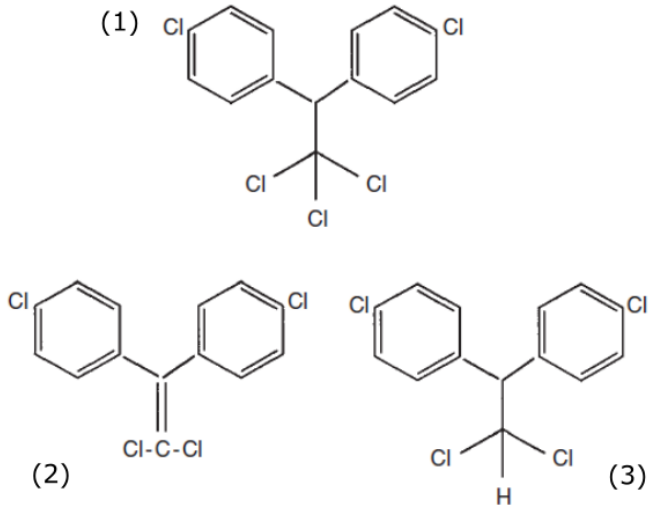

Figure 4. Structures of *p,p′*-DDT (1), *p,p′*-DDE (2) and *p,p′*-DDD (3).

DDT is the best known and one of the most widely used pesticides in the world, with an estimated production around 50 million kg per year in the mid-1950's, especially because of its wide spectrum, long-lasting properties, and low cost in comparison to arsenicals and other inorganic insecticides (Matsumura, 2009). It was estimated that during the first decade of use (1940 – 1950) DDT saved 5 million lives and prevented 100 million illnesses related to malaria, typhus, etc., while in the agricultural sector DDT is considered a major advance allowing the development of the modern agriculture (Kumar De, 2006).

While DDT was being massively used worldwide, the first ecological concerns regarding its persistence in the environment and bioaccumulation started to be presented, especially with the publication of "Silent Spring" in 1962 where Rachel Carlson observed the bioaccumulation of DDT in bird eggs (Rosner and Lauterstein, 2009). DDT has been banned in the northern hemisphere and regulated worldwide since the 1970s; Sweden banned DDT in 1970, the USA in 1972, Italy in 1978, and the UK in 1986 (Binelli and Provini, 2003; Rogan and Chen, 2005). After around four decades of restrictions and gradual reducing in its usage, DDT is still in use in some countries in the Southern Hemisphere, though current world usage is small (~1kt y^{-1}) compared with historical use (>40kt y^{-1}) from 1950 to 1980 (de Boer et al., 2008; Geisz et al., 2008).

- *PAHs*

Polycyclic aromatic hydrocarbons (PAH) are considered to be priority pollutants and as such knowledge of their distribution is of significant environmental concern (Morris et al., 1994). A wide variety of PAHs are ubiquitously found in the environment mostly as a result of the incomplete combustion of organic material (pyrolytic PAHs) and storm runoff, industrial discharges and petroleum spills (petrogenic PAHs) (Alebic-Juretic, 2011). Although PAHs can originate from both natural processes (e.g. forest-fires and natural petroleum seeps) and anthropogenic activities, most PAH input in the environment is related to the anthropogenic activity that is generally considered to be the major source of these compounds (e.g. traffic, industry, incineration processes, petroleum production and transportation) (Cachada et al., 2012; Samanta et al., 2002; Soclo et al., 2000).

Each source (pyrolytic and petrogenic hydrocarbons) gives rise to characteristic PAH pattern, and it is therefore possible to get access to the processes that generate the compounds (Soclo et al., 2000). In general, petrogenic PAHs present 2 to 3 aromatic rings in their structures and the presence of alkyl-substituted homologues is common, while pyrolytic PAHs present higher molecular weight with 4–6 aromatic rings (Figure 5; Colombo et al., 1989; Yunker et al., 2002; Hu et al., 2010). Thus, contamination origin can be assessed through different methods including the ratio between PAHs, e.g. ratios between phenanthrene and anthracene (Phe/Ant and Ant/Ant+Phe), and the ratio between low- molecular weight PAHs (2-3 aromatic rings) and high-molecular weight PAHs (4-6 aromatic rings; LMW and HMW, respectively) (Martins et al., 2007; Yunker et al., 2002).

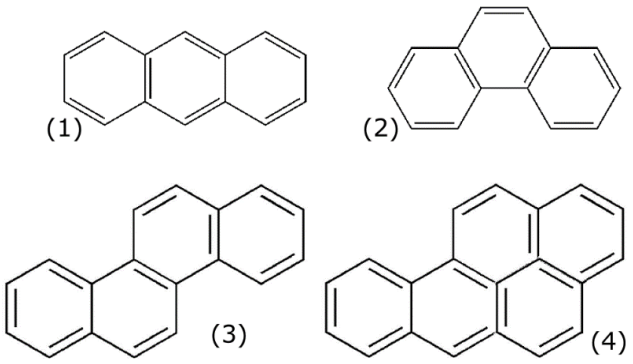

Figure 5. Structure of some low-molecular weight PAHs (1) anthracene and (2) phenanthrene and high-molecular weight PAHs (3) chrysene and (4) benzo[a]pyrene.

Although PAHs as not listed in the Stockholm Convention on POPs, they are recognized as POPs under the Aarhus Protocol (Lohmann et al., 2007) and as priority substances for environmental monitoring by European Directives (e.g. Directives 2013/39/EU and 2008/56/EC – MSFD; European Comission, 2008, 2013; Crise et al., 2015). Sixteen PAHs have been specified by the United States Environmental Protection Agency (EPA) as priority pollutants and are usually used to evaluate anthropogenic pollution levels in environment: naphthalene, acenaphthylene, acenaphthene, fluorene, anthracene, phenanthrene, pyrene, fluoranthene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene, indeno[1,2,3-cd]pyrene, benzo[g,h,i]perylene (Ruiz-Fernández et al., 2012; Stevens et al., 2003). Environmental risks associated with these compounds are related to the high toxicity and carcinogenic character of several individual hydrocarbons (such as benzo[a]pyrene); thus, understanding the pollution levels of PAHs and their source identification is important in terms of assessing the environmental status of a system (Fu et al., 2003; Samanta et al., 2002).

1.1.2. Emerging contaminants

In contrast to the "classical" contaminants, emerging contaminants (or contaminants of emerging concern – CECs), are chemical compounds that are not or are only partly regulated and the knowledge on their occurrence, fate and effects is still limited (Laane et al., 2013). Most of these synthetic products containing chemicals of emerging concern are not necessarily new in the market, but are not included in routine monitoring programs (Pintado-Herrera et al., 2013; Tijani et al., 2015). Thus, many of these chemicals have likely been present in the environment for several years or even decades, but they were not previously detectable using available analytical methodologies (Maruya et al., 2014).

CECs encompass a vast number of compounds, including personal care products (PCPs), such as fragrances, UV-filters, insect repellents, and antimicrobials, organophosphorus compounds, pharmaceuticals, endocrine disrupting compounds and nanoparticles (Maruya et al., 2014; Pintado-Herrera et al., 2016). Fragrances are used in

a broad range of products, including perfumes, shampoos and detergents. The synthetic musks are one of the most widely used class of fragrances and encompasses three chemical groups – nitro-musks, polycyclic musks and macrocyclic musks (Sumner et al., 2010). The two most important commercial synthetic musks are tonalide (AHTN) and galaxolide (HHCB; Figure 6), which are polycyclic musks used extensively as perfuming agents in a vast array of household and PCPs (Casatta et al., 2015).

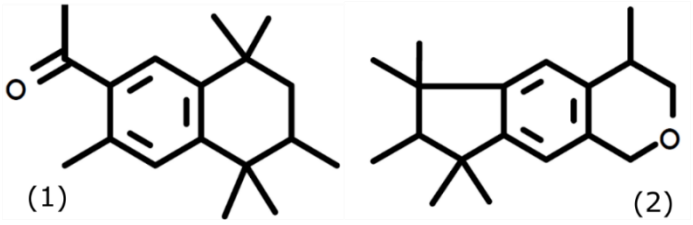

Figure 6. Tonalide (1, AHTN) and galaxolide (2, HHCB)

Similarly, UV filters are also applied in various PCPs such as sunscreens, cosmetics, body washes and hair sprays with the purpose to provide protection against damage by UVA and UVB radiation (Ramos et al., 2015; Tsui et al., 2015). These compounds can be either organic (chemical) absorbers or inorganic (physical) blockers, depending on the basis of their mechanism of action. Organic UV filters absorb UV radiation and screen out UV rays by changing them into less harmful energy while inorganic blockers (commonly known as sunblock) form a barrier that would block UV rays from reaching the skin (Ramos et al., 2015). Organic UV filters include many groups of compounds, such as benzophenone and its derivatives (e.g. 2-Hydroxy-4-methoxybenzophenone – BP3 and 2-Hydroxy-4-methoxybenzophenone-5-sulfonic acid – BP4; Figure 7) and crylene derivatives (e.g. 2-ethylhexyl 2-cyano-3,3-diphenylacrylate – octocrylene).

Emerging contaminants are extensively used in everyday life in consumer goods, household activities, industry and agriculture, being continuously introduced to sewage waters (Gros et al., 2008). However, most wastewater-treatment plants (WWTPs) are not designed to treat these types of substances: some studies found that the removal of certain CECs at treatment plants could be as low as ~10-20% (Du et al., 2014; Petrović et al., 2003). Thus, one of the main sources of CECs to aquatic systems are untreated urban wastewaters and WWTP effluents (Gros et al., 2006).

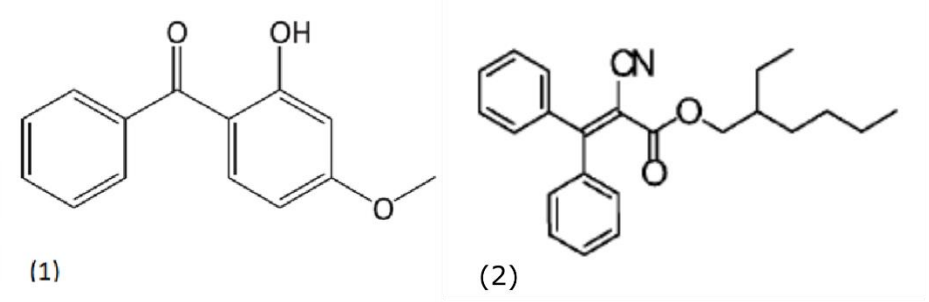

Figure 7. BP3 (1) and octocrylene (2).

Physicochemical properties of these compounds will determine their behavior and fate in environment. In general, fragrances and UV filters not considered as volatile compounds (boiling points ~ 400° C) and some of them may be moderately or highly soluble in water (e.g. BP3and BP4, respectively) or very low soluble in water (e.g. octocrylene, galaxolide, tonalide), which means that are not likely to be found in water bodies (Ramos et al., 2015 and references therein). Another factor controlling environmental distribution of these compounds is the octanol-water partition coefficient (log K_{ow}). For instance, octocrylene and galaxolide are more hydrophobic than BP3 (log K_{ow}=5.9, 6.88 and 3.79, respectively; Pintado-Herrera et al., 2013), which means they will rather accumulate in fat tissue or adsorb to particulate matter than being present in the aqueous phase.

Although CECs generally do not stay in the environment as long as POPs, they are defined as "pseudo persistent" because their high transformation and removal rates can be offset by their continuous introduction into the environment, exposing humans and biota as if they were persistent (Casatta et al., 2015; Petrović et al., 2003). Currently, the knowledge about UV filters and fragrances occurrence and ecotoxicity is scarce. However, recent studies detected possible adverse effects related to these compounds. For instance, BP3 provoked deformities and increasing rate of coral bleaching on coral planulae even at low concentrations, being considered a skeletal endocrine disruptor and hazard to coral reef conservation (Downs et al., 2015); some UV filters, including BP3 and octocrylene, presented potential to accumulate through the trophic food chain in Norway (Langford et al., 2015); and high levels of galaxolide and tonalide were found in human adipose tissues in Italy (Schiavone et al., 2010).

Despite the fact that CECs are usually found in relatively low concentrations in environmental matrixes, the long-term risks that the presence of a large variety of chemicals may pose for non-target organisms as well as for human health is still poorly known (Gros et al., 2006; Tijani et al., 2015). This is especially concerning because most of these compounds are not yet regulated and depending on their fate and behavior in WWTPs, drinking water treatment plants and aquatic systems, the probability of biota and human exposure to them is considerable high (Tijani et al., 2015).

1.2. Study area

The Adriatic Sea (Figure 8) is a shallow semi-enclosed basin (approximately 200 km wide and 800 km long) connected to the Mediterranean Sea through the Strait of Otranto (Gomiero et al., 2011; Manca et al., 2002). The area can be divided into three sub-basins: the northern Adriatic, at the north of Ancona and with depths up to 100 m; the middle Adriatic, between Ancona and the Gargano Promontory, reaching depths until 270 m; and the southern Adriatic, from the Pelagosa Sill to the Otranto Strait, which includes the deepest area of the Adriatic Sea (the South Adriatic Pit, up to 1200 m; Artegiani et al., 1997; Turchetto et al., 2007).

The main water masses in the Adriatic Sea are: Adriatic Surface Water (ASW), the less saline and particle rich water flowing on the western Italian side; the Levantine Intermediate Water (LIW), coming from the eastern Mediterranean with high salinity (~38.70); the North Adriatic Deep Water (NAdDW), formed over the northern shelf and the densest water of the whole Mediterranean; and the South Adriatic Deep Water (SADW), formed in the southern Adriatic (Artegiani et al., 1997; Manca et al., 2002; Turchetto et al., 2007).

Freshwater input comes mainly from the Po River (in the northern Adriatic), which is responsible for the transport of approximately one-fourth of the material that enters the Adriatic Sea (Frignani et al., 2005). The Po delta includes five distributary mouths (the Maestra, Pila, Tolle, Gnocca and Goro), draining large agricultural and industrial areas, inhabited by 15 million of people (Romano et al., 2013; Tesi et al., 2007; Viganò et al., 2015).

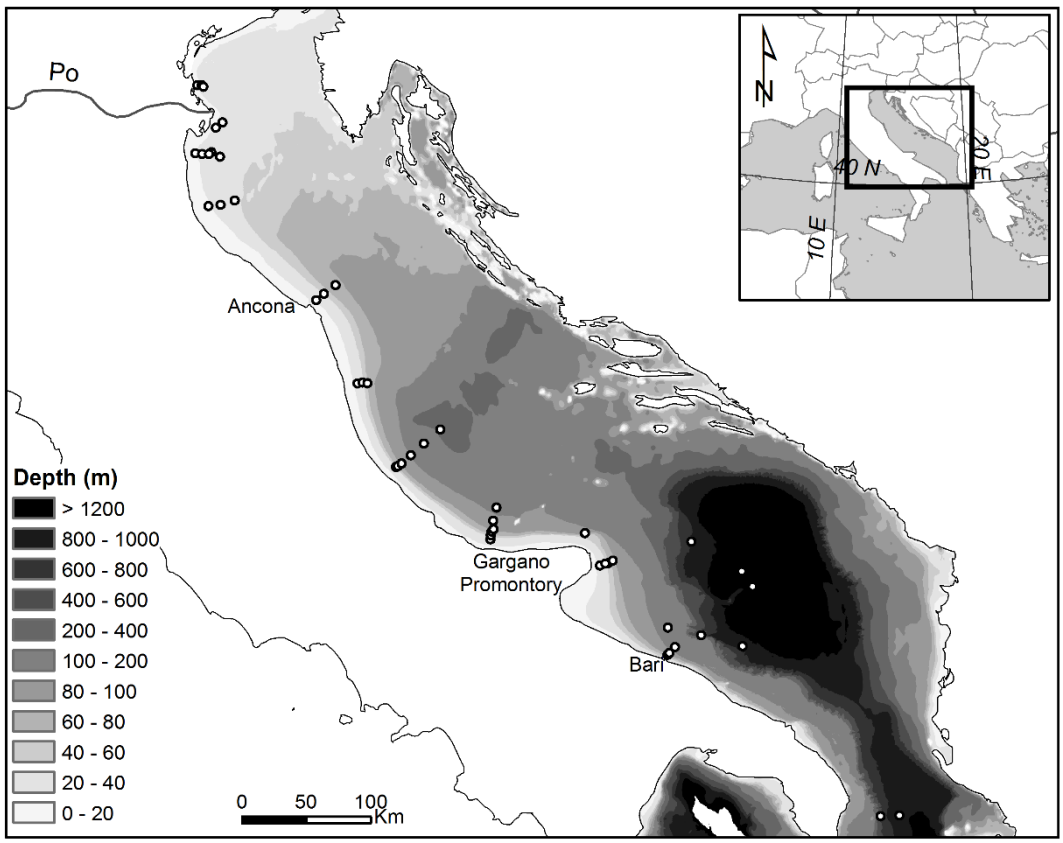

Figure 8. Map and location of the Adriatic Sea.

The Po River is the largest river in Italy, with approximately 650 km of extension and a drainage basin of more than 70,000 km^2 (Frignani et al., 2005; Viganò et al., 2015). According to data obtained from the Italian Regional Agency for Environmental Protection and Control (ARPA), the mean discharge to the Adriatic Sea is 1500 m^{3}s^{-1}, with minimum and maximum annual discharges of ~800 and ~2600 m^{3}s^{-1} (Figure 9). The hydrograph of the Po is characterized by two seasonal increases in discharge (caused by spring snow melt and autumn rainfall) and episodic high-discharge events, when the mean discharge can be as high as 8,000 m^{3}s^{-1} (Palinkas and Nittrouer, 2007).

In general, the water circulation as well as the transport of materials in the Adriatic basin is very dependent on thermohaline factors, resulting in counter-clockwise flow (Figure 10.1) that varies seasonally and intensified currents along the western Adriatic coast (Artegiani et al., 1997; Palinkas and Nittrouer, 2007). The water circulation has three major components: river runoff, characterized by the low salinity waters derived mainly from the Po River; wind and heat forcing at the surface, which produce deep-

water masses in the northern and southern Adriatic and forces the circulation to be seasonal; and the Otranto Channel forcing, which inputs heat and salt in the circulation as a restoring mechanism for the northern heat (Artegiani et al., 1997).

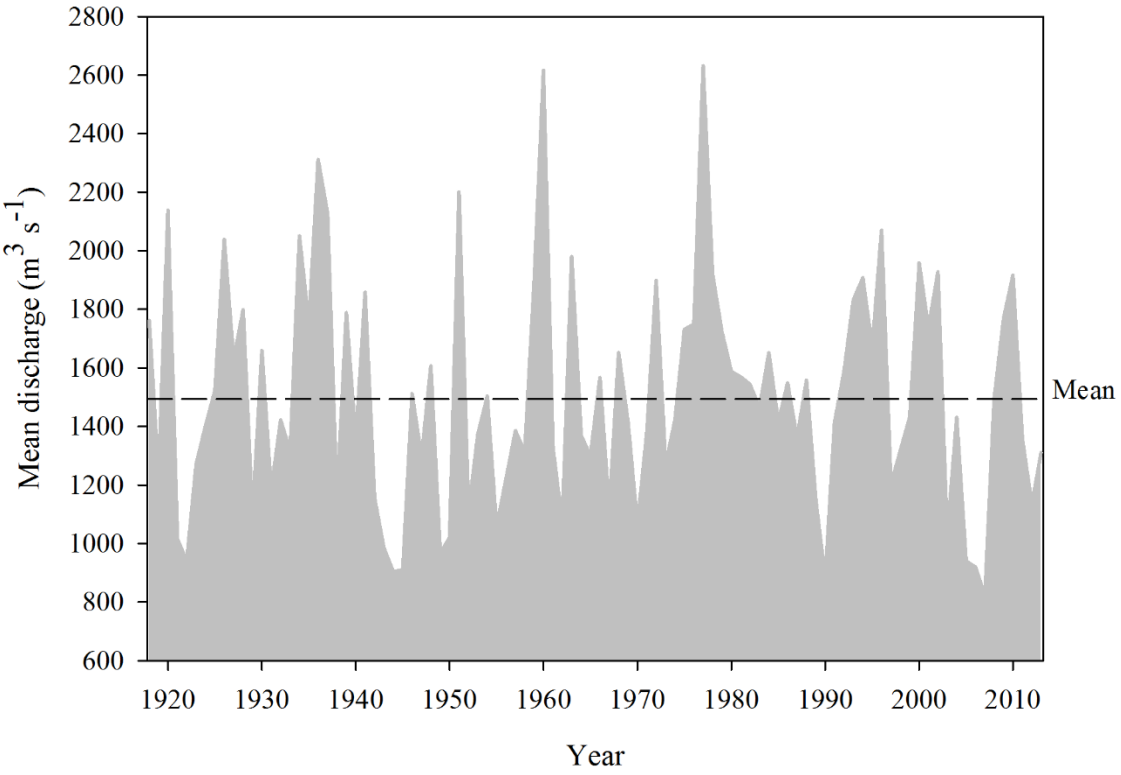

Figure 9. Po Rivers mean annual water discharge (m^3s^{-1}) to the Adriatic Sea. The dashed line represents the mean river discharge from 1918 to 2013 (1500 m^3s^{-1}).

As a result of the oceanographic process in the Adriatic Sea, riverborne material is distributed southwards and accumulates in a continuous belt of deltaic and shallow-marine deposits forms the late-Holocence mud wedge along the western Adriatic shelf (Frignani et al., 2005; Tesi et al., 2013). The late Holocene clinoform, shown in Figure 10.2 and 10.3, reaches up to 35 m in thickness with a volume of almost two hundred cubic kilometers and sedimentation outside this clinoform is negligible and not recognizable using seismic profile (Cattaneo et al., 2003; Tesi et al., 2013).

Another possible area for material accumulation in the Adriatic Sea is the Bari Canyon System (BCS). The BCS is located in the South-Western Adriatic Margin (SWAM), that shelters the deepest areas in the Adriatic Sea, including also the South Adriatic Pit, the Gondola Slide and the Dauno Seamont (Carniel et al., 2015; Langone et al., 2015;

Trincardi et al., 2007). The BCS probably represents the conduit through which the sediment derived from the Po River (~600 km far away) approaches the ultimate repository, potentially capturing sediment in transit and transporting it into the southern Adriatic basin (Turchetto et al., 2007). The major mechanism influencing this process is the cascading of the North Adriatic Dense Water (NAdDW) in the area (Tesi et al., 2008; Turchetto et al., 2007), which in particular periods is able to quickly transfer suspended particles coming from the North Adriatic (Langone et al., 2015).

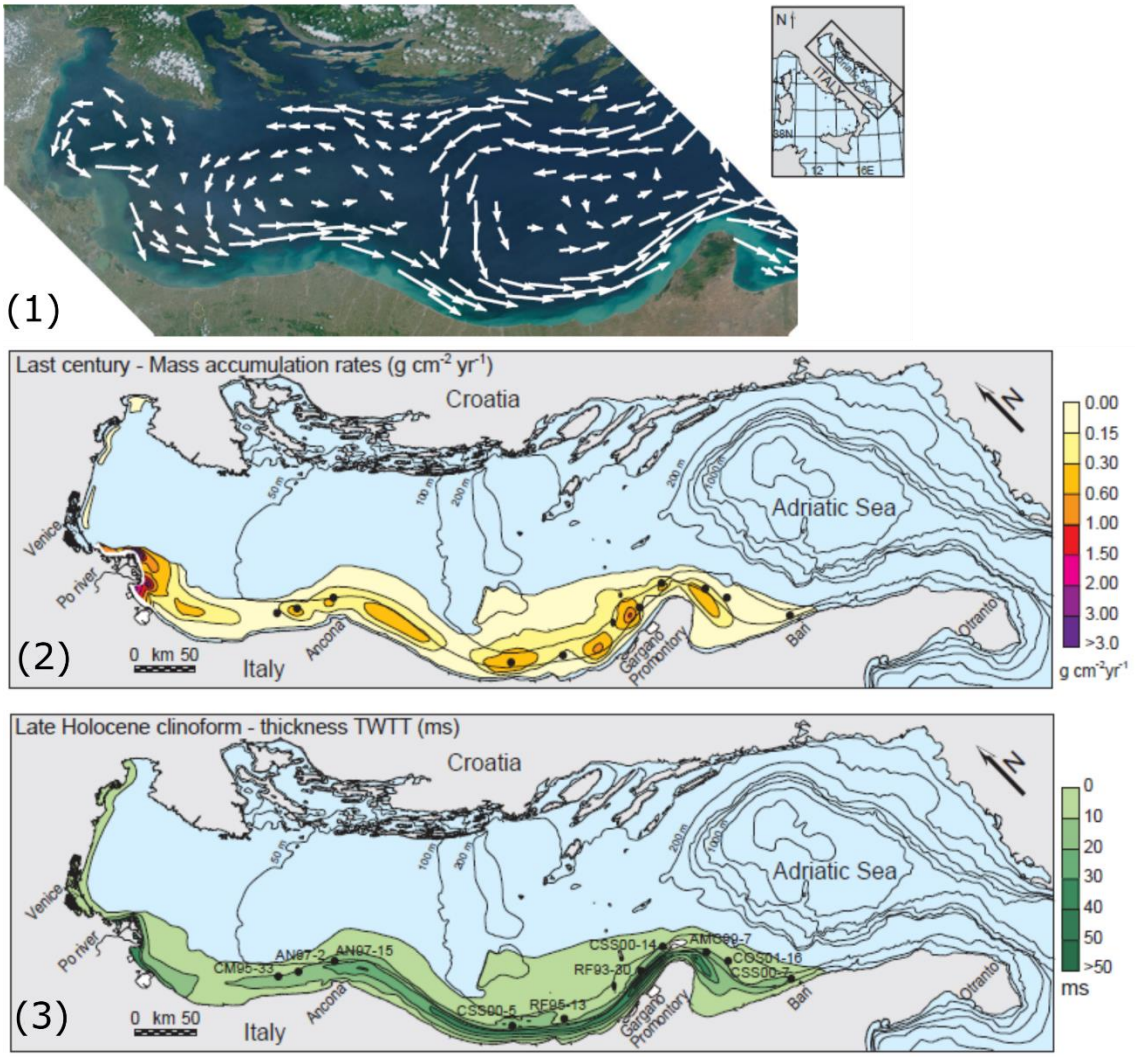

Figure 10. Map of the Adriatic Sea: (1) surface circulation is indicated by the white arrows; (2) mass accumulation rates (g cm^{-2} yr^{-1}) within the clinoform; and (3) thickness of the clinoform (Cattaneo et al., 2004).

1.3. Objectives and thesis summary

Human activities and influences are intensive in the Adriatic Sea, which presents heavily industrialized, urbanized and agriculturally productive coastal areas (Romano et al., 2013) that may represent important sources of contamination to this sea basin. Marine systems are usually the final reservoirs for contaminants, where sediments represent a sink for most pollutants (Sahu et al., 2009). Some contaminants deposited in sediments have the potential to accumulate through the food web, affecting marine biota, aquatic-dependent wildlife, and ultimately human health (Borgå et al., 2001; Mourier et al., 2014). Hence, the anthropic pressures in the Adriatic Sea affect this marine environment as a whole.

The major sources of contaminants in the western Adriatic Sea have been related to coastal industrial activities as well as riverine discharges, especially associated with the Po River input, which receives influences from highly urbanized and industrialized areas from northern Italy (De Lazzari et al., 2004; Guzzella and Paolis, 1994; Viganò et al., 2015). Previous studies have suggested that the ultimate repository for these contaminants are deep-sea areas located in the southern Adriatic Sea (especially the Bari Canyon), where the cascading of the North Adriatic Dense Water (NAdDW) would be able to quickly transfer suspended particles (and, therefore, particle-binding contaminants) coming from the North Adriatic (Turchetto et al., 2007; Tesi et al., 2008; Langone et al., in press).

Thus, this doctorate thesis was based on the following working hypotheses:

- Contaminants concentrations in sediments should decrease southwards in the western Adriatic Sea as a result of the influence of the Po River as the major source of contaminants in the area;
- Particle-binding contaminants should be present in deep-sea sediments from the southern Adriatic, confirming the transfer of contaminants from coastal areas to deep-sea areas under the influence of the cascading of the NAdDW;
- Sediment cores may provide information on the trends and chronology of pollution for legacy and emerging contaminants in the western Adriatic Sea.

Despite the availability of an important amount of data sets and long time series for several contaminants families, there is still lack of data for specific contaminants, especially regarding contaminants of emerging concern. For legacy contaminants, most of the data available refers to restricted areas and a lack of off-shore datasets have been detected (Crise et al., 2015). A wide-ranging work monitoring diferent groups of contaminants in sediments is a key tool for a comprehensive understanding of contaminants behavior and identification of the overall environmental quality and possible threatens to the whole marine ecosystem.

This work is a part of FP7 PERSEUS (Policy-oriented marine Environmental research in the Southern European Seas), which aims to identify the interacting patterns of natural and human-derived pressures on the Mediterranean and Black Seas, linking them to the MSFD descriptors, criteria and indicators. In this context, the aims of this work were:

(a) Investigate levels and spatial patterns of selected groups of legacy and emerging contaminants in sediments from the western Adriatic Sea;
(b) Evaluate the occurrance and concentrations of these contaminants in deep-sea sediments in order to provide information on their transfer from coastal areas to the open sea along the western Adriatic margin;
(c) Assess the historical trends of the contaminants in sediment cores from the western Adriatic Sea to track the historical escalation of human pressures over this area, and to associate the contaminants tendencies with the historical use and emissions of these compounds;
(d) Identify the major potential sources of these contaminants in the western Adriatic Sea based on the pollutants behavior and distribution.

The present doctoral thesis is divided in four chapters. *Chapter 1* presented an introduction to the topic, with a general description of the target compounds and their behavior in the marine environment, as well as the study area and the main aims of this work. *Chapter 2* presents the field and laboratory methods adopted in the present work. This chapter is divided in four subsections, addressing the sampling procedures (section 2.1); the analyses of the sediment characteristics (section 2.2); the analytical methodologies for PCBs (section 2.3) and for the concomitant analyses of legacy and emerging compounds (section 2.4), including the extraction, clean-up and instrumental

analyses. *Chapter 3* consists of three manuscripts, which present the results and discussion of this work. The first article is entitled "*Polychlorinated biphenyls (PCBs) in sediments from the western Adriatic Sea: sources, historical trends and inventories*" and focuses on the concentrations and behavior of PCBs, discussing historical records and major sources of these compounds to the western Adriatic Sea. The second article is entitled "*Fate and distribution of legacy and emerging contaminants along the western Adriatic Sea*" and encompasses a broader range of pollutants, focusing especially on their sources and spatial distribution along the whole Adriatic Sea. The third article is entitled "Sedimentary record of PCBs, DDTs and PAHs in coastal and deep-sea sediments from the Adriatic Sea (Italy)" and presents a discussion comparing the vertical trends of legacy contaminants, assessing possible sources and transfer of these compounds from coastal to deep-sea areas in the Adriatic Sea. Finally, *Chapter 4* presents briefly and concisely the main conclusions arising from this thesis.

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Chapter 2

Methodology

Preamble

This work has been designed in order to gain information about not only pollutants concentrations and accumulation in the sediments of the western Adriatic Sea, but also to address potential contamination sources and spatial and historical trends at a basin level. The sampling design and the oceanographic cruises were accomplished under the coordination of the Consiglio Nazionale delle Ricerche - Istituto di Scienze Marine (CNR-ISMAR) from Bologna, Italy. The joint plan was to better characterize the origin, transfer, pathways and fate of pollutants in the Southern Adriatic Sea, in strict connection with activities planned in PERSEUS WP2 (Pressures and Impacts at Coastal Level) in the Northern Adriatic Sea. Of particular interest was get a picture of the distribution of pollutants in the Po river dispersion system from the prodelta area to the Southern Adriatic, through the analysis of surface sediments and sediment cores. In order to achieve the proposed objectives, this work has been divided in two main parts, as follows:

(1) PCB congeners analyses

The first part of the present thesis is focused on the analysis of polychlorinated biphenyls (PCBs) in sediment cores and recent sediments in order to investigate potential sources, historical trends and inventories of these compounds. Samples were collected in five transects along the north-south axis of the western Adriatic Sea: Po River prodelta, Ancona, the Gargano Promontory, Bari and at the mud waves fields in deep marine systems from the southern Adriatic.

(2) Emerging and legacy contaminants analyses

The second part of this work covers a larger set of compounds, including legacy (e.g. PAHs, PCBs and DDTs) and emerging contaminants (e.g. fragrances and UV filters). Levels and spatial patters of these chemicals were assessed in sediments from the western Adriatic Sea in an attempt to fill the gap regarding data on emerging contaminants and transfer of contaminants from coastal waters to the open sea.

Sediment samples were collected from Trieste to Otranto during the oceanographic campaign “ADREX 14”.

2.1. Sampling

The sampling plans were designed based on detailed morpho-bathymetric maps available (Trincardi et al., 2014) and taking into account the average sedimentation rates for the last century from reference data (Alvisi, 2009; Frignani et al., 2005; Palinkas and Nittrouer, 2007). Sediments were collected along the Adriatic mud-wedge in transversal-to-the-coast transects from northern to southern Adriatic Sea. Sediment samples were also taken at the mud waves fields in deep marine systems from the southern Adriatic (mud waves field named A and B, located north of the Gondola slide and northwest of Dauno seamount, respectively) in order to assess at what extent land-derived pollutants are accumulated in the southern Adriatic basin, which is currently unknown.

For the first part of this work, sediments were collected along five transversal-to-the-coast transects on board the R/V Urania in April 2013 (southern Adriatic), R/V Dallaporta in November 2013 (central and southern Adriatic) and R/V Urania on February 2014 (northern Adriatic). Undisturbed sediment cores (one key-station for each transect) were retrieved in the Po River prodelta (core J25), Ancona (core AN2), the Gargano Promontory (core GG2), Bari (core BA5) and in the mud waves field named A located north of the Gondola slide (core DE15bis).

For the second part of this work, a sediment sampling was performed in October 2014 on board the O/V OGS Explora in numerous transversal-to-the-coast transects from Northern to Southern Adriatic (from Trieste to Otranto). Several surface sediment samples were collected along the Adriatic mud wedge and in the surrounding areas of the Bari Canyon. Sediment cores were retrieved in the Po River prodelta (station 9), off the Bari Canyon (station 1) and on a sediment drift in the Bari Canyon (station 2). The complete cruise report (Langone et al., 2014) is presented in Appendix B.

Surface sediments were taken by a mini box corer or oceanic box corer and the top 0.5-cm of undisturbed sediment was collected (Figure 11). Sediment cores (length ≤ 60 cm; diameter: 10 cm) were collected using a cylindrical box-corer or the gravity sediment

corer SW104, specially designed to preserve the sediment-water interface, and sectioned onboard at 1-cm intervals. All samples were placed into pre-cleaned glass jars with aluminum foil liners on the lid to avoid potential leaching, and stored at -20 °C until processing and analysis.

Figure 11. Sediment cores and surface sediment sampling.

2.2. Sediment characteristics

The sediment characteristics analyses were conducted at the Consiglio Nazionale delle Ricerche - Istituto di Scienze Marine (CNR-ISMAR) from Bologna, Italy.

2.2.1 Porosity, grain size and elemental analyses

In the laboratory, all sediment layers were weighted, oven-dried at 55°C to constant weight. The samples were re-weighted and water content was determined by calculating the amount of weight lost during the drying procedure. Porosity (ϕ) was calculated from the loss of water between wet and dry sediment according to equations suggested by Berner (1971), assuming a sediment density of 2.6 g cm^{-3} and a water density of 1.034 g cm^{-3}.

For the grain size determination, homogenized sediment samples were pretreated for ~72-96 hours with H_2O_2 (30%) in order to eliminate the organic matter content. After this pretreatment, the samples were wet sieved at 63 μm to separate sands from silt and clay fractions. The sands are collected in a beaker and oven-dried at 55°C to constant weight. The fine fraction (silt and clay) was allowed to settling for ~72 hours. After the particles were settled, the supernatant water was discarded and the fine fraction was oven-dried at 55°C to constant weight. The grain size distribution was assessed as the ratio between the weight obtained for each fraction and the initial weight of the total sample.

The elemental analyses followed the procedure described by Tesi et al. (2007). Briefly, sediment samples were first decarbonated after acid treatment (1.5M HCl). Total and organic carbon (OC) and total nitrogen (TN) content were measured on a Fison CHNS-O Analyzer EA 1108. The average standard deviation (SD) of each measurement, determined by replicate analyses of the same sample, was ±0.07% and ±0.01% for OC and TN, respectively. Stable isotopic analyses of organic C ($\delta^{13}C$) were carried out on the same samples using a FINNIGAN Delta Plus mass spectrometer directly coupled to the FISONS NA2000 EA by means of a CONFLO interface for continuous flow measurements. All isotopic data were expressed in the conventional delta notation (%):$\delta^{13}C$ $C_{sample} = (R_{sample}/R_{reference} - 1) \times 1{,}000$ with $R = {}^{13}C/{}^{12}C$, relative to the Peedee belemnite standard (PDB). Total inorganic carbon (TIC) was calculated from the difference between TC and TOC, and then converted in $CaCO_3$ using 8.33 as atomic conversion factor.

2.2.2 Estimated date

Sediment accumulation rates (SARs) and mass accumulation rates (MARs) based on radioisotope geochronology (mainly ^{210}Pb and ^{137}Cs) were extensively assessed in the Adriatic Sea. Accordingly, different datasets were combined based on triangle-based linear interpolation in order to obtain better spatial distribution (Frignani et al., 2005; Palinkas and Nittrouer, 2007; Tesi et al., 2013). Since information on accumulation rates and strata chronologies in the deep Adriatic is scarce, sediment cores sampled in this are (DE15bis, station 1 and station 2) were measured for ^{210}Pb activities. Alpha

counting of daughter isotope ^{210}Po, considered in secular equilibrium with its grandparent Ra^{226}, was used for ^{210}Pb analyses.

In order to estimate the date for each section of the sediment cores, the sediment accumulation rate reported for each key-station was used, as follows:

$$\text{Estimated date [}\textit{anno Domini}\text{ (A.D.)]} = a - \left(\frac{b}{c}\right)$$

where *a* is the year in which the core was collected, *b* is the depth of the section in the core and *c* is the SAR of each core.

2.3. PCB congeners analyses

The quantification of PCBs in environmental samples is performed through several steps, such as extraction, purification, and instrumental analysis. A range of issues can affect the efficiency of environmental chemistry analysis, such as the usage of large volumes of solvents and reagents, which in some cases are toxic (e.g. toluene and dichloromethane for the extraction of organic compounds), low sensitivity and selectivity, long extraction times, and high costs (Zhang et al., 2011). Within all the analytical steps, the extraction is of major importance since it is responsible for the transfer of the compounds from the matrix of interest to the solvent.

In this work, two extraction techniques were tested in order to choose the most appropriate one for the analyses of PCB congeners in the sediment samples. Soxhlet extraction and accelerated solvent extraction (ASE) were tested and compared for recovering of PCBs in spiked marine sediment samples from the western Adriatic Sea. The tests were conducted at the Centro Ricerche e Servizi Ambientali (C.R.S.A., MED Ingegneria, Marina di Ravenna, Italy), and the results were presented during the international event ECOMONDO (www.ecomondo.com) in Rimini, Italy, on November 2014. The extended abstract with the detailed information on the tests and the results is presented in the Appendix C.

Briefly, both methods, ASE and Sohxlet extraction, were tested in triplicates and followed the same clean-up methodology and instrumental analyses. Sohxlet extraction presented relatively higher recovery in comparison to the ASE (95 ± 14% and 76 ±

22%, respectively). However, a loss of some low-chlorinated congeners was observed during ASE extraction. For this reason, the Sohxlet technique was selected for the analyses of PCB congeners in sediments from the Adriatic Sea.

One of the most important issues regarding contaminant analysis is the amount of chemicals currently or historically used. Estimates suggest that more than 100,000 chemicals are currently in use for different purposes around the world (Robles-Molina et al., 2014). The existence of such a high number of compounds in the environment leads to interferences during chemical analyses, reducing the efficiency on the detection of target compounds (Liu et al., 2006). This is especially true for PCBs, which can be present in low concentrations in environmental samples (ppb or ppt), and whose identification can be hindered or mislead by the presence of overlapping peaks of compounds such as phthalates and other chlorinated molecules. To this aim, a strong clean-up procedure with acidic silica chromatographic column, which is used to remove lipids and other interferences from the sample extract, has been adopted.

2.3.1 Analytical method

Sediment samples were air-dried under a fume hood and then homogenized using a blender. Approximately 10 g of dried sediments were Sohxlet extracted using a mixture of acetone and n-hexane (20:80) for 16 hours. The samples were placed in pre-cleaned cellulose thimbles and TCMX (tetrachloro-m-xylene, AccuStandard, USA) was added as surrogate standard. Clean-up and fractionation of the extracts was accomplished through passage on an acidic silica gel column (Figure 12; 30% H_2SO_4) and activated copper powder was used to eliminate interfering sulphur compounds (adapted from US EPA, 2008). PCBs were eluted with 60 ml of a dichloromethane (DCM) and n-hexane mixture (40:60). The extracts were concentrated to a final volume of 1 mL under a gentle gas stream of purified nitrogen. PCB 198 (AccuStandard, USA) was added to the samples prior to the injection as internal standard.

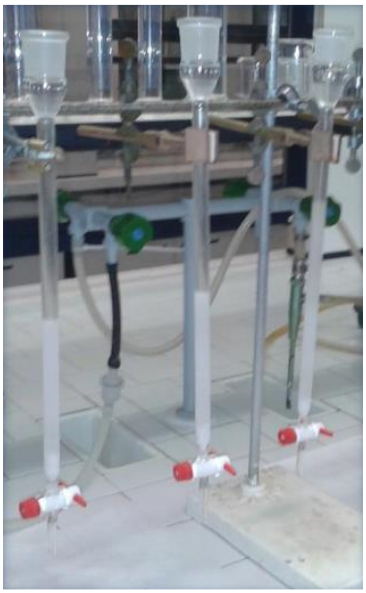

Figure 12. Acidic silica gel column chromatography for PCB congeners clean-up.

2.3.2 Instrumental Analyses

The determination of PCB congeners was performed by gas chromatography (GC Varian CP 3800) with electron capture detection (ECD) equipped with an MDN-5S column (length: 30 m, ID: 0.25 mm, film thickness: 0.25 μm). The oven temperature started with 100 °C for 2 minutes, increasing of 15°C per minute until 160°C and increasing 5°C until 270°C with a final hold time of 10 minutes. The equipment calibration was made with a calibration curve prepared with a PCB standard (C-WNN, AccuStandard, USA, 28 congeners: PCB 8, PCB18, PCB 28, PCB 52, PCB 44, PCB 66, PCB 101, PCB 81, PCB 77, PCB 123, PCB 118, PCB 114, PCB 153, PCB 105, PCB 138, PCB 126, PCB 187, PCB 128, PCB 167, PCB 156, PCB 157, PCB 180, PCB 169, PCB 170, PCB 189, PCB 195, PCB 206, PCB 209) at the following concentrations: 1, 2, 5, 10 and 20 ng mL^{-1}. The analytes were identified by comparison of the retention times of the peaks detected in each replicate with the peaks obtained on the calibration curves. The quantification of the PCBs was based on the area obtained for each analyte in the samples, according to the mass/area ratio obtained for the internal standard and on the response factor obtained from the calibration curve. The results were confirmed by GC/MS (mass spectrometry) analysis. Spike tests were performed by analyzing a replicate sediment from a reference site spiked with the PCB standard. The mean standard recovery in the spike tests was 94.0 ± 12.4 % (Figure 13), with mean relative standard deviation (RSD) of 14 ± 5.3 %.

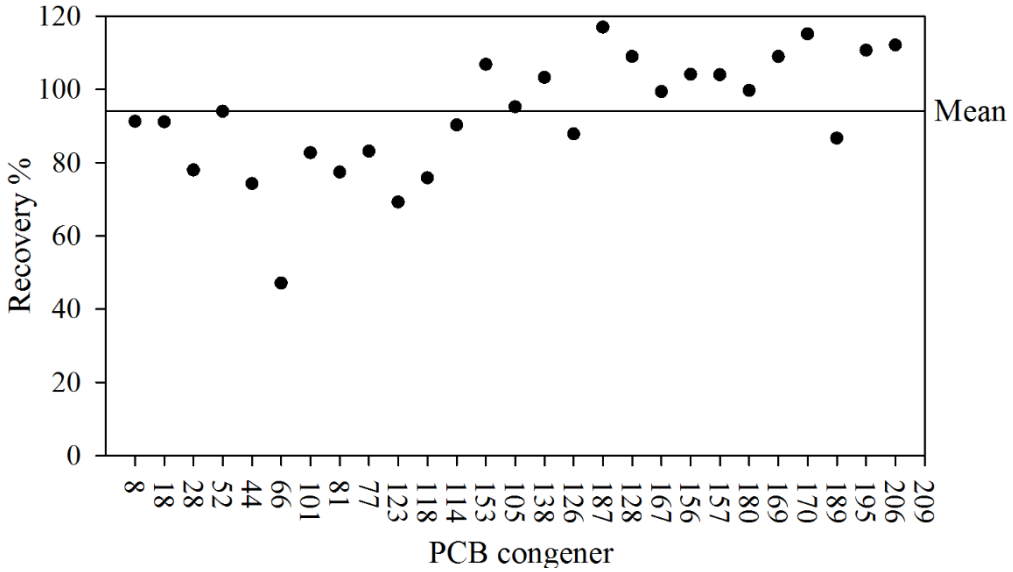

Figure 13. Mean PCB standard recovery in the spike tests (n=7).

2.4. Emerging and legacy contaminant analyses

The development and optimizations of analytical methods that are environmental friendly and capable of detecting multiple classes of compounds at very low levels (e.g. parts per trillion – ppt) is currently one of the biggest challenges in environmental chemistry (Lara-Martín et al., 2011; Pintado-Herrera et al., 2016, 2013). In recent decades, many advances have occurred in this sense, with the advent of new analytical techniques and the improvement of instrumental analyses. For instance, new techniques have been developed for the extraction of legacy contaminants, such as PAHs and PCBs, *in tandem* with emerging contaminants and/or for the elimination of the clean-up step (Dimitriou-Christidis et al., 2015; Pintado-Herrera et al., 2016, 2013; Robles-Molina et al., 2014; Subedi et al., 2015).

The analytical procedures regarding this step of the work were conducted at the Physical Chemistry Department, Faculty of Marine and Environmental Sciences, University of Cadiz (Cádiz, Spain), under the supervision of Prof. Pablo Antonio Lara-Martín, based on the methodology optimized by Pintado-Herrera et al. (2016).

2.4.1 Analytical method

Sediment samples were extracted using an accelerated solvent extraction ASE 200 system (Figure 14a; Dionex, USA). Briefly, the extraction cells (Figure 14b; 11 mL) were prepared with 1 g of activated silica (150°C for 16 hours) and 0.5 g of activated copper powder. Approximately 4 g of air-dried and milled sediment (Figure 14c) were homogenized with 1g of alumina and placed into the extraction cells. The extraction procedure consisted of three static extraction cycles, where the samples were pre-heated for 5 minutes and extracted for 5 minutes in each cycle at a temperature of 100°C and a pressure of 1500 psi. After the three cycles, the cell was rinsed with dichloromethane (flush volume: 60% of cell volume) and purged with N_2 stream for 60 s.

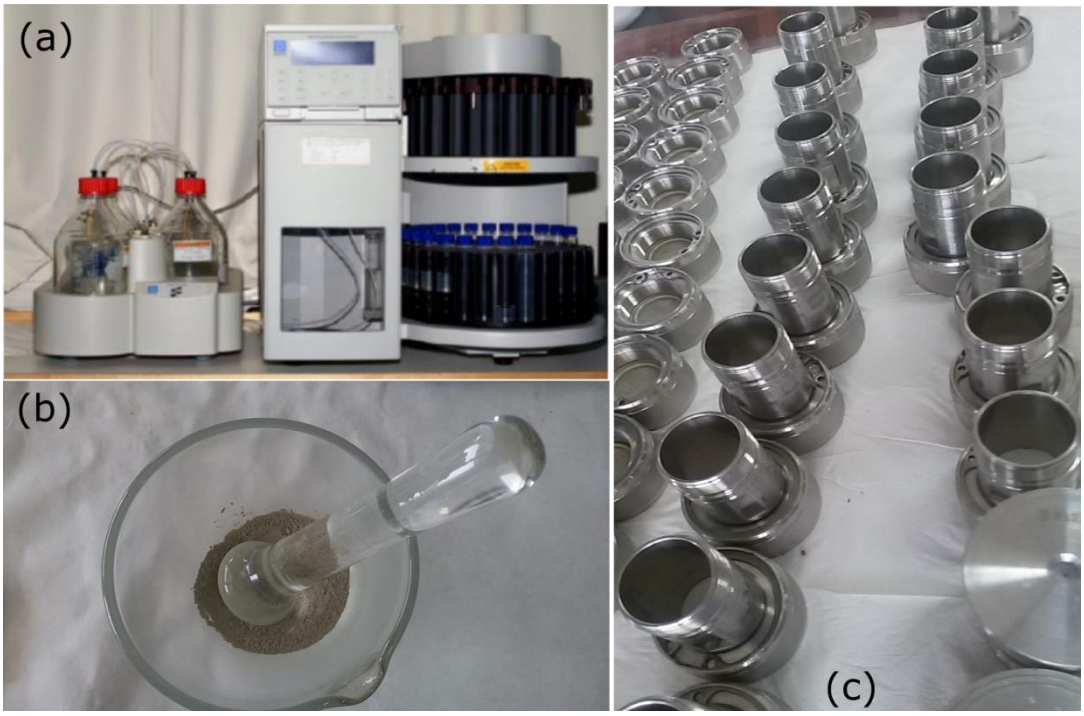

Figure 14. (a) accelerated solvent extraction ASE 200 system; (b) sediment samples preparation; and (c) ASE extraction cells (11 mL).

The eluates were evaporated to dryness and re-dissolved in 0.5 mL of ethyl acetate. The final extracts were centrifuged (10,000 rpm for 10 minutes) to remove possible interferences and kept under refrigeration until the chromatographic determinations. Procedural blanks were performed for each extraction series of 10 samples using alumina and analyzed in the same way as samples. Method detection limits (MDL) were

determined for each analyte as 3 times the signal to noise ratio in spiked sediment samples.

2.4.2 Instrumental analyses

Separation, identification and quantification were performed using gas chromatography (Figure 15; SCION 456-GC, Bruker) coupled to a triple quadrupole mass spectrometer equipped with a BR-5ms column (length: 30 m, ID: 0.25 mm, film thickness: 0.25 μm). The oven temperature was programmed to 70 ºC for 3.5 min, increasing at 25 ºC min-1 to 180 ºC, increasing at 10 ºC min-1 to 300 ºC, holding this temperature for 4 min. A derivatizing agent (MTBSTFA) and internal standards (mixture of deuterated compounds) were added to the samples prior to the injection. Calibration curves were prepared for each target compound at different concentrations (from 5 to 500 ng g^{-1}). Target compounds were identified and quantified by comparison of retention times and two transitions of each analyte (one for quantification and one for confirmation) of the samples with external standard solutions.

Figure 15. Gas chromatograph coupled to triple quadrupole mass detector (GC/MS/MS).

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CHAPTER 3

Results and discussion

Preamble

This chapter contains the results and discussion related to the present doctoral thesis, which are presented over three manuscripts:

(1) The first manuscript is entitled "Polychlorinated biphenyls (PCBs) in sediments from the western Adriatic Sea: sources, historical trends and inventories" and has been submitted (revised version) to the journal Science of the Total Environment (Impact factor: 4.099). This manuscript provides the first extensive data set on the historical and current levels and inventories of PCBs in sediments from the western Adriatic Sea. Additionally, it identifies the main potential sources of PCBs in the region and provides evidences to the hypothesis of PCBs transfer from anthropized areas in the north to the deep Adriatic Sea by the cascading of the North Adriatic Dense Water (NAdDW).

(2) The second manuscript, entitled "Fate and distribution of legacy and emerging contaminants along the western Adriatic Sea" has been submitted and is currently Under Review by the journal Environmental Pollution (Impact factor: 4.143). This manuscript provides unprecedented data on occurrence, levels and spatial patterns of emerging contaminants such as UV filters and fragrances (e.g. tonalide, galaxolide, octocrylene, benzophenone-3 and OTNE) in sediments from the western Adriatic Sea. It also contains the first extensive dataset on the distribution of legacy contaminants (PAHs, DDTs and PCBs) over the entire western Adriatic basin.

(3) The third manuscript entitled "Sedimentary record of PCBs, DDTs and PAHs in coastal and deep-sea sediments from the Adriatic Sea (Italy)" in under preparation and will be submitted to the journal Marine Pollution Bulletin (Impact factor: 2.991). This manuscript aims to understand the transfer of contaminants from coastal areas to open sea sediments along the western Adriatic margin and reconstruct the historical inputs and estimate the deposition fluxes of selected classes of POPs in sediment cores from: (a) a coastal area

nearby a highly industrialized region in the north of Italy (Po River prodelta) and (b) a deep-sea area at the South-Western Adriatic Margin (SWAM).

Article I

Polychlorinated biphenyls (PCBs) in sediments from the western Adriatic Sea: sources, historical trends and inventories

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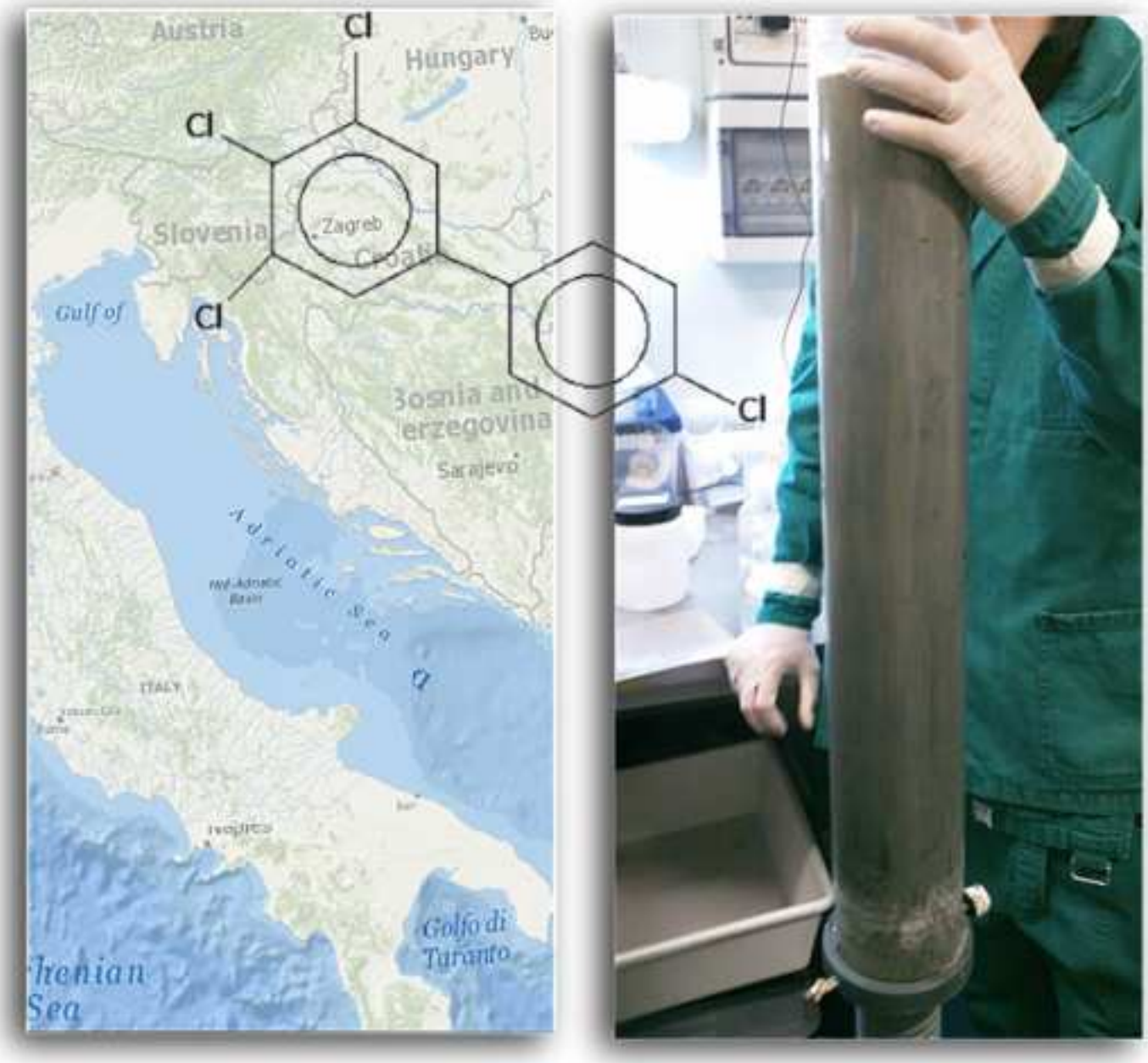
***Graphical Abstract**



Highlights

- PCBs were determined in recent and dated sediments from the western Adriatic Sea
- Unprecedented data of PCB levels, historical trends and inventories were assessed
- PCBs historical trends corresponded to their production and use over time
- Congener composition indicate the Po River as the major contributor of PCBs
- High-chlorinated PCBs in the deeper Adriatic suggest influence of cascading process

Abstract

Sources, historical trends and inventories of polychlorinated biphenyls (PCBs) were investigated in sediments collected in five transects along the north-south axis of the western Adriatic Sea. The concentration of total PCBs ($\sum_{28}$ PCBs) ranged from <LOD (limit of detection) to 9.0 ng g^{-1} in the sediment cores and between 0.1 and 2.2 ng g^{-1} in recent sediments. Chronological records of PCB concentrations displayed a common pattern with historical PCB production and use, with the maximum peak values detected between the 1960's and the 1980's. Sediments deposited within the last two decades presented a ~40% to ~80% PCBs reduction in comparison to the peak levels, reflecting the ban on PCB production and use since the late 1970's. PCB levels along with the presence of high-chlorinated congeners decreased southwards, indicating the Po River as the major source of PCBs in the western Adriatic Sea. This is further corroborated by the estimated inventories of PCBs, which were ~ 4-7 times higher in the Po River prodelta (256 ng cm^{-2}) in comparison to the middle and southern Adriatic, respectively, and about 100 times higher than the in the deep Adriatic Sea.

Keywords: Persistent organic pollutants, sediment cores, homologue patterns, fluxes, Adriatic mud-wedge

1. Introduction

Polychlorinated biphenyls (PCBs) are a classical group of Persistent Organic Pollutants (POPs) that were extensively used worldwide since they were first produced, in 1930. Due to their physical-chemical properties, PCBs were mainly used as electric fluids in transformers and capacitors, hydraulic lubricants and flame retardants, and to a lesser extent, in plasticizers, carbonless copy paper, paints, among numerous other applications (Fiedler, 1997; Borja et al., 2005). Global production of PCBs was estimated to exceeded 1.3 million tons and Italy is among the major consuming countries corresponding to over 2% of global PCB consumption (Breivik et al., 2007). PCBs production and usage have been globally restricted since the 1970's because of their adverse effects, including endocrine disrupting and carcinogenic effects and biomagnification properties (Borgå et al., 2001; Frignani et al., 2007).

In spite of the production and use ban, PCBs are still in use in closed systems, especially in electrical equipment. Consequently, PCBs can still be detected in the environment, and their current levels are not expected to decrease significantly within the next few decades (K Breivik et al., 2007; Sobek et al., 2015). Currently, PCBs can reach the environment by urban and industrial sewage discharge, leaching from contaminated soils, direct spillages into soils, urban runoff and volatilization (Breivik et al., 2002b; Litskas et al., 2012). PCBs are mainly transported from sources by atmosphere and water bodies to the open sea, where sediments usually represent their final sink (Ruiz-Fernández et al., 2012; Argiriadis et al., 2014).

The Adriatic Sea is a shallow semi-enclosed basin connected to the Mediterranean Sea through the Strait of Otranto (Manca et al., 2002). Human activities and their influences are intensive in the area pressuring the Adriatic marine ecosystems. Consequently, the Adriatic Sea is an important and interesting area for pollution studies sheltering heavily industrialized, urbanized and agriculturally productive areas (Dujmov et al., 1993). The major sources of POPs have been related to coastal industrial activities as well as riverine discharges, especially associated with the Po River, which is the largest and most important Italian river (Galassi and Provini, 1981; Guzzella and De Paolis, 1994; De Lazzari et al., 2004). In the Adriatic Sea, the dispersion of riverborne materials and associated pollutants is driven by the general cyclonic water circulation and

oceanographic conditions. Consequently, fine sediments accumulate in a belt parallel to the Italian coast (Correggiari et al., 2001; Frignani et al., 2005).

There is little data related to the occurrence and levels of PCBs in sediments from the Adriatic Sea, which has been observed since the 1990's (Fowler et al., 2000; De Lazzari et al., 2004; Pozo et al., 2009). Although there is plenty of information regarding contaminants concentrations in coastal and riverine systems in the Adriatic Sea (Galassi and Provini, 1981; Acquavita et al., 2014; Guerra et al., 2014; Viganó et al., 2015), marine sediments have been studied to a lesser extension and usually within delimited regions (e.g. Caricchia et al., 1993; Fowler et al., 2000). A wide-ranging work is fundamental for a comprehensive understanding of the extension and patterns of PCBs at the Adriatic Sea basin level, providing tools to identify the evolution of anthropic pressures and possible threats to the Adriatic ecosystem as a whole.

The aim of this work is to assess historical patterns, inventories and potential sources of PCB in sediments along the western Adriatic Sea. This work is part of the PERSEUS EU FP7 Project (Policy-oriented marine Environmental research in the Southern European Seas), which aims to provide information on pressures and impacts considered as major threats to the good environmental status of the marine systems, addressing them to the Marine Strategy Framework Directive (MSFD) descriptors.

2. Material and methods

2.1. Sampling

Sediments were collected on the mud-wedge along five transversal-to-the-coast transects placed from northern to southern Adriatic Sea on board the R/V Urania in April 2013 (southern Adriatic), R/V Dallaporta in November 2013 (central and southern Adriatic) and R/V Urania on February 2014 (northern Adriatic). Undisturbed sediment cores (one key-station for each transect) were retrieved in the Po River prodelta (core J25), Ancona (core AN2), the Gargano Promontory (core GG2), Bari (core BA5) and the Gondola slide (core DE15bis) (Figure 1).

Sediment cores (length $\leq$ 50 cm; diameter: 10 cm) were collected using a cylindrical box-corer or the gravity sediment corer SW104, specially designed to preserve the sediment-water interface, and sectioned onboard at 1-cm intervals. Surface sediments

were taken by a mini box corer or oceanic box corer and the top 0.5-cm of undisturbed sediment was collected. All samples were placed into pre-cleaned glass jars with aluminum foil liners on the lid to avoid potential leaching, and stored at -20 °C until processing and analysis.

2.2. Sediment characteristics and estimated date

In the laboratory, all sediment layers were weighed, oven-dried at 55°C, and then re-weighed to determine water content. Porosity (ϕ) was calculated from the loss of water between wet and dry sediment according to equations suggested by Berner (1971), assuming a sediment density of 2.6 g cm^{-3} and a water density of 1.034 g cm^{-3}. Grain size was determined after a pre-treatment with H_2O_2 and wet sieving at 63 μm to separate sands from silt and clay fractions. Total and organic carbon (OC) and total nitrogen (TN) content were measured on a Fison CHNS-O Analyzer EA 1108. Samples for OC analysis were first decarbonated after acid treatment (1.5M HCl). Based on the analysis of replicate samples, the average standard deviation (SD) of the results was ±0.07 and ±0.01% for OC and TN, respectively (Tesi et al., 2007).

Sediment accumulation rates (SARs) and mass accumulation rates (MARs) based on radioisotope geochronology (mainly ^{210}Pb and ^{137}Cs) were extensively assessed in the Adriatic Sea. Accordingly, different datasets were combined based on triangle-based linear interpolation in order to obtain better spatial distribution (Frignani et al., 2005; Palinkas and Nittrouer, 2007; Tesi et al., 2013). Since information on accumulation rates and strata chronologies in the deep Adriatic is scarce, sediment core sampled in the Gondola site (core DE15bis) was measured for ^{210}Pb activities. Alpha counting of daughter isotope ^{210}Po, considered in secular equilibrium with its grandparent Ra^{226}, was used for ^{210}Pb analyses. Estimated SAR for each key-station is reported in Table 1. The highest SARs were observed in the Po River prodelta (0.79± 0.12 cm y^{-1}) where sediments accumulate preferentially in two depocenters (Tesi et al., 2013). Other important sites of deposition are Ancona (AN2; 0.35± 0.04 cm y^{-1}) and the Gargano promontory (GG2; 0.46± 0.06 cm y^{-1}).

In order to estimate the date for each section of the sediment cores, the sediment accumulation rate reported for each key-station was used, as follows:

129 $$\text{Estimated date [}\textit{anno Domini}\text{ (A.D.)]} = a - \left(\frac{b}{c}\right)$$

130 where *a* is the year in which the core was collected, *b* is the depth of the section in the
131 core and *c* is the SAR of each core (Martins et al., 2014).

132

133 *2.3. PCBs analyses*

134 Sediment samples were air-dried under a fume hood and then homogenized using a
135 blender. Approximately 10 g of dried sediments were placed in pre-cleaned cellulose
136 thimbles and TCMX (tetrachloro-m-xylene, AccuStandard, USA) was added as
137 surrogate standard. Samples were Sohxlet extracted using a mixture of acetone and n-
138 hexane (20:80) for 16 hours. Clean-up and fractionation of the extracts was
139 accomplished through passage on an acidic silica gel column (30% H_2SO_4) and
140 activated copper powder was used to eliminate interfering sulphur compounds (adapted
141 from US EPA, 2008). PCBs were eluted with 60 mL of a dichloromethane (DCM) and
142 n-hexane mixture (40:60). The extracts were concentrated to a final volume of 1 mL
143 under a gentle gas stream of purified nitrogen. PCB 198 (AccuStandard, USA) was
144 added to the samples prior to the injection as internal standard.

145 The determination of PCBs was performed by gas chromatography (GC Varian CP
146 3800) with electron capture detection (ECD) equipped with a Supelco MDN-5S column
147 (length: 30 m, ID: 0.25 mm, film thickness: 0.25 μm). The oven temperature started
148 with 100 °C for 2 minutes, increasing of 15°C per minute until 160°C and increasing
149 5°C until 270°C with a final hold time of 10 minutes. The equipment calibration was
150 made with a calibration curve prepared with a PCB standard (C-WNN, AccuStandard,
151 USA, 28 congeners: PCB 8, PCB18, PCB 28, PCB 52, PCB 44, PCB 66, PCB 101,
152 PCB 81, PCB 77, PCB 123, PCB 118, PCB 114, PCB 153, PCB 105, PCB 138, PCB
153 126, PCB 187, PCB 128, PCB 167, PCB 156, PCB 157, PCB 180, PCB 169, PCB 170,
154 PCB 189, PCB 195, PCB 206, PCB 209) at the following concentrations: 1, 2, 5, 10 and
155 20 ng mL^{-1}. The analytes were identified by comparison of the retention times of the
156 peaks detected in each replicate with the peaks obtained on the calibration curves. The
157 quantification of the PCBs was based on the area obtained for each analyte in the
158 samples, according to the mass/area ratio obtained for the internal standard and on the
159 response factor obtained from the calibration curve. Confirmatory analysis of selected

PCB peaks was accomplished by GC/MS analysis (Agilent HP 5970 MSD interfaced to an HP 5890 GC). Concentrations of single PCB congeners and $\sum_{28}$ PCBs are given in ng g^{-1} sediment dry weight.

2.4. Quality-Assurance Procedures

The quality-assurance procedures included precision tests, analyses of procedural blanks and matrix spikes (Wade and Cantillo, 1994). The mean surrogate recovery, based on the relationship with the internal standard (PCB 198) added before the GC-ECD analyses, was of 90.0 ± 13.8 %. Spike tests were performed by analyzing a replicate sediment from a reference site spiked with the PCB standard. The mean standard recovery in the spike tests was 94.0 ± 12.7 %, with mean relative standard deviation (RSD) of 12.4 ± 5.1 %. A procedural blank was performed for each extraction batch using 10 g of anhydrous sodium sulphate heated to 450°C prior to extraction and analyzed in the same way as the samples. The limit of detection (LOD) was calculated as the average blank value plus three times the standard deviation of the blanks, and varied between 0.01 and 0.26 ng g^{-1} (Table S1).

3. Results and discussion

3.1. Sediment characteristics

Summary results regarding grain size distribution, OC and OC/TN atomic ratio are presented in Table 1 for sediment cores and Table 2 for surface sediments. Fine fractions (silt and clay) were predominant in sampled sediments accounting for 73.3 - 99.8%. Grain size data are comparatively uniform and consistent with other studies accomplished within the western Adriatic mud-wedge (Frignani et al., 2005; Cattaneo et al., 2007; Tesi et al., 2007, 2013 and references therein; Romano et al., 2013), where the sediment released to by the Po and Apennine rivers consists primarily of (> 90 percent) silt and clay particles (i.e., smaller than 64 μm) (Nittrouer et al., 2004). OC exhibited a relatively low content and a limited variability in the mud-wedge sediments from the Adriatic Sea, varying between 0.4 and 1.3% (0.7 ± 0.1%).

These levels are consistent with previous data on OC content found in sediments from the western Adriatic Sea (Tesi et al., 2007; Turchetto et al., 2007; Tesi et al., 2013). In general, OC and C/N ratio presented a decreasing trend from northern to southern Adriatic Sea, with mean OC values of 1.2 ± 0.04 and 0.5 ± 0.06% and mean C/N ratios of 11 ± 0.4 and 8.4 ± 0.7 from the Po River prodelta (core J25) to the Gondola Slide (core DE15bis), respectively.

3.2. PCBs levels and trends

Individual and total PCBs concentrations (ng g^{-1}) in surface samples and sediment cores are available in the Supplementary material (Tables S1 – S6). The concentration of total PCBs ($\sum_{28}$ PCBs) ranged between 0.1 and 2.2 ng g^{-1} in surface sediments from Ancona (AN1, AN2, AN3), the Gargano promontory (GG1, GG2), Bari (BA4, BA5, BA6), and the Gondola slide (from DE1 to DE21), and from <LOD to 9.0 ng g^{-1} in the sediment cores (J25, AN2, GG2, BA5, and DE15bis). In general, $\sum_{28}$ PCBs concentrations in surface samples decreased seaward with water depth, with the maximum concentration detected much closer to the coast in Ancona (AN1, 2.2 ng g^{-1}). The concentrations found between the 60 – 80 m isobaths (sediments AN3, BA4, BA5 and GG1) were very similar, with a mean concentration of 1.4 ± 0.2 ng g^{-1}.

$\sum_{28}$ PCBs concentrations also decreased southwards. The highest concentrations were detected in the sediment core close to the Po River prodelta (J25) in the top 1-cm layer and at 20 cm depth (5.2 and 9.0 ng g^{-1}, respectively). In the southern Adriatic (represented by key-stations in the Gargano Promontory and Bari), $\sum_{28}$ PCBs concentrations ranged from 0.3 to 2.2 and from <LOD – 3.2 ng g^{-1} (cores GG2 and BA5, respectively). The lowest concentrations were detected around the mud waves field located on the north of the Gondola slide (DE01 to DE21), where total PCB concentrations ranged from 0.1 to 0.3 ng g^{-1} for surface sediments and from <LOD to 1.4 ng g^{-1} for the sediment core DE15bis.

The concentrations detected in recent surface sediments in this study present a significant decrease in comparison to those previously detected in northern and middle Adriatic (Caricchia et al., 1993; Fowler et al., 2000) and in the eastern Adriatic (Picer and Picer, 1991), reflecting the restrictions on use and productions of these compounds (Table 3). In general, total PCB concentrations detected in the southern Adriatic were

similar to those obtained for deep sediments of the eastern Mediterranean Sea (Mandalakis et al., 2014) and in coastal areas from Spain (Eljarrat et al., 2005; Solé et al., 2013. Total PCB concentrations in the sediment core collected close to the Po River prodelta (J25) were similar to those previously reported in the northern Adriatic (Fowler et al., 2000; De Lazzari et al., 2014), but lower than those registered in more industrialized and urbanized areas, such as the Mar Piccolo of Taranto (Cardellicchio et al., 2007), the East China Sea (Yang et al., 2012), the Baltic Sea (Sobek et al., 2015), and the Salton Sea (Sapozhnikova et al., 2004).

Gómez-Gutiérrez et al. (2007) proposed background levels of contamination for the Mediterranean Sea. With reference to PCBs contaminants, these levels were established as 1 – 5 ng g^{-1} (median 2 ng g^{-1}) as the sum of seven indicator PCB congeners ($\sum_7$ PCBs: PCBs 28, 52, 101, 118, 138, 153, and 180). $\sum_7$ PCBs levels were above the background levels only in the Po River prodelta (sediment core J25), where 70% of the sediment samples exceeded the median background level, and the $\sum_7$ PCBs concentrations ranged from 1.0 to 5.5 ng g^{-1}. Concerning the environmental quality standards (EQS), the Italian Decree n. 260/2010 (D.M. 260/2010) set an EQS of 8 ng g^{-1} for PCB in sediments of transitional and coastal environments ($\sum_{13}$ PCB 28, 52, 77, 81, 101, 118, 126, 128, 138, 153, 156, 169, 180). The $\sum_{13}$ PCBs levels detected in this work did not exceed, in any sediment core, the proposed EQS. Further studies considering additional parameters (e.g. other classes of contaminants) are warranted to the estimation and evaluation of possible ecotoxicological risks in sediments from the western Adriatic Sea.

3.3. PCB composition and sources

As the physico-chemical properties of PCBs are dependent on their degree of chlorination (Fiedler, 1997), the analysis of PCB congeners and homologue profiles provide useful insights on the behavior and the possible sources of PCBs to the marine environment (Mai et al., 2005; Barakat et al., 2013).

In general, dominant PCB congeners were PCB 81, PCB 8, PCB 209, PCB 187 and PCB 118 (Tables S1 – S6 from Supplementary material). While congener composition was considerably different among the sediment cores, surface samples displayed an analogous predominance of low-chlorinated congeners (especially PCB 8 and PCB 81).

This pattern indicates the same origin of PCBs and low influence of local input sources in recent sediments (Tolosa et al., 1995; Barakat et al., 2013). Low-chlorinated PCBs are more volatile and less persistent in the marine environment, being usually related to long-range transport and/or dechlorination processes, while the higher chlorinated compounds are associated to local input sources and contaminated areas (Tolosa et al., 1995; Borja et al., 2005).

Differences among areas in the western Adriatic Sea, tested by one-way ANOVA, were significant for PCBs homologues composition (F-ratio = 6.7, p value < 0.05). The tetra-chlorinated biphenyls (tetra-CB) were the most prevalent homologues in sediment from Bari, the Gargano Promontory and the Gondola slide (cores BA5, GG2 and DE15bis), accounting for ~70%, 60% and 40% of the total PCBs, respectively. Conversely, penta- and hepta-chlorinated biphenyls were the dominant homologues in Ancona (core AN2) and Po River prodelta (core J25), representing ~40% and 30% of the total PCBs, respectively. Different homologue composition among sediment cores may be related to variable inputs of PCB mixtures to the western Adriatic sea (Fowler et al., 2000).

Higher abundance of the low-chlorinated congeners (di-, tri-, tetra- and penta-CB) were detected in middle and southern Adriatic, with an average contribution ranging from ~60% to nearly 100% in sediment cores from Bari, the Gargano Promontory and the Gondola Slide (BA5, GG2 and DE15bis) (Figure 3). In general, the contribution of high-chlorinated congeners (hexa- to deca-CB) decreased southwards, from ~50% in the Po River prodelta (core J25) to ~2-3% in the Gargano Promontory (core GG2). The decreasing patterns of total and particularly higher-chlorinated PCBs from the northern to the southern Adriatic Sea and the prevalence of lower-chlorinated congeners in the southern cores suggest a predominant influence of the Po River discharge in the Northern Adriatic. The Po River has a drainage basin of 75,000 km^2 being one of the main drainage basins in Europe. It extends eastward across northern Italy and receives influences from highly urbanized and industrialized areas thus representing one of the major sources of PCBs and other contaminants to the Adriatic Sea (Guzzella and Paolis, 1994; Tesi et al., 2007;Viganó et al., 2015).

Even though low-chlorinated congeners were predominant in southern Adriatic, the presence of some high-chlorinated PCBs found within the Gondola slide, especially hexa- and hepta-CB (PCB 138 and PCB 157), may indicate local input sources. Considering that these samples were taken from a remote, deep-sea area (500 to 600 m

depth), the presence of PCBs sources seems to be unlikely. A possible explanation for the presence of high-chlorinated congeners is the influence of the cascading of the North Adriatic Dense Water (NAdDW) in the area (Turchetto et al., 2007; Tesi et al., 2008), which in particular periods is able to quickly transfer suspended particles coming from the North Adriatic (Langone et al., 2015). The NAdDW is the densest water of the whole Mediterranean, formed over the Adriatic northern shelf during cold months and spreading southward along the western Italian shelf reaching the southern Adriatic basin (Vilibić and Supić, 2005). The intensity of the NAdDW cascading depends on numerous factors (e.g. atmospheric temperature; precipitation; bottom morphology), playing a first order control on the particulate fluxes through the western margin of the Southern Adriatic (Langone et al., 2015).

Although reductive dechlorination of PCBs can occur in anoxic environments, leading to transformation of PCB congener composition from higher to lower-chlorinated compounds over time (Zoumis et al., 2001; Borja et al., 2005; Sobek et al., 2015), downcore distribution of low- and high-chlorinated PCBs were rather constant within cores and no shift on homologue composition was evident; hence, dechlorination processes are not likely to occur in the sediments of the western Adriatic Sea (Figure 1 of Supplementary material).

3.4. Historical records of PCBs

Downcore variation of $\sum_{28}$ PCBs concentrations (ng g^{-1}) in the sediments from the western Adriatic Sea is showed in Figure 2. Annual PCBs fluxes (ng cm^{-2} y^{-1}) were estimated as follows:

$$\text{Flux} = C_i r \rho_i$$

where C_i is the concentration of $\sum_{28}$PCBs in sediment layer i (ng g^{-1}), r is the SAR for each sediment core (cm y^{-1}) and ρ_i is the dry mass of the sediment layer i (g cm^{-3}; Mai et al., 2005).

Calculated PCBs fluxes followed the same patterns as those detected for $\sum_{28}$PCBs concentrations (Figure 2). Briefly, the bottom layers of the sediment cores (sediments deposited before 1940) registered the lower mean PCB fluxes, ranging from 0.01 ng cm^{-2} y^{-1} in Ancona (core AN2) to 4.3 ng cm^{-2} y^{-1} in the Po River prodelta (core J25). PCB fluxes were fairly low and constant until the middle of the 1960's, where mean values

ranged from 0.3 ± 0.1 ng cm^{-2} y^{-1} offshore the Gargano Promontory (core GG2) to 4.2 ± 0.4 ng cm^{-2} y^{-1} in the Po prodelta (core J25). Maximum values of 4.7 ng cm^{-2} y^{-1}, 1.3 ng cm^{-2} y^{-1}, 1.1 ng cm^{-2} y^{-} and 0.8 ^{1} ng cm^{-2} y^{-}were detected between the 1970's and the early 1990's in the Po River prodelta, Ancona, the Gargano Promontory and Bari, respectively; a subsequent decrease reaching back constant levels of ~ 2-3 ng cm^{-2} y^{-1} in the Po River prodelta (core J25), ~ 0.4 ng cm^{-2} y^{-1} in Ancona and Bari (cores AN2 and BA5), and ~ 0.3 in the Gargano Promontory (core GG2) occurred in the last two decades.

Historical trends of total PCB ($\sum_{28}$ PCBs) showed a common pattern, with increasing concentrations from the lower horizons to the middle sections of sediment cores from the Po River prodelta, Ancona, the Gargano Promontory and Bari followed by a decreasing trend upwards in the surface layers deposited in recent years (Figure 2). Sediments from the Gondola Slide (core DE15bis) present particular characteristics (e.g. distance from the coast and non-detectable or negligible PCB concentrations), that makes it unsuitable for the reconstruction of PCB historical record. Therefore, these data will not be discussed for PCBs historical records hereafter.

Low concentrations of total PCBs ($\sum_{28}$ PCBs: 0.1 – 3.5 ng g^{-1}) were detected in sediments deposited prior to the period of first PCB production (before 1930). Similar observations for PCB concentrations were extensively reported in previous works (e.g. Mai et al., 2005; Frignani et al., 2007; Piazza et al., 2009; Mugnai et al., 2011) and were ascribed to a number of factors, such as physical mixing of sediments, bioturbation, and/or downward migration of PCB congeners in the sediment column. Similarly to the results reported by Mai et al. (2005), low-chlorinated PCBs were in general more abundant in sediments deposited before 1930 in the western Adriatic Sea, suggesting preferable post-depositional mobilization of lighter congeners.

After the first detection of PCBs, historical concentrations showed an increasing trend until the middle of the 1950's in the Adriatic Sea sediments from the Po River prodelta (core J25), Ancona (core AN2) and Bari (core BA5) except in the Gargano Promontory (core GG2), following the escalation of PCBs use after their production started.

In the Po River prodelta sediments (core J25), a PCBs peak concentration of 8.1 ng g^{-1} was detected at the beginning of the 1950's, corresponding to a major Po River's flood occurred in November 1951. According to data obtained from the Italian Regional Agency for Environmental Protection and Control (ARPA), a mean discharge close to

8,000 m^3s^{-1} was registered during this heavy flood event. Large floods can mobilize upstream PCB sources, resulting in inputs of contaminated sediments, and thus affect PCBs distribution (Mourier et al., 2014).

Maximum peak concentrations of 9.0 ng g^{-1}, 3.9 ng g^{-1}, 2.2 ng g^{-1}, and 3.2 ng g^{-1} occurred between 1960's and the 1980's in the Po River prodelta (core J25), Ancona (core AN2), the Gargano Promontory (core GG2) and Bari (core BA5), respectively, coinciding with the maximum PCB production worldwide and with the predicted trends on PCB consumption and emission in Italy (Breivik et al., 2002b; Breivik et al., 2007) (Figure 4). This period also corresponds to the beginning of the production of PCB by the Caffaro industry in northern Italy (Breivik et al., 2002b).

In the Po River prodelta sediments, high-chlorinated PCBs predominates especially between the 1970's and the mid 1980's, accounting for approximately 50% of the total PCBs mixture ($\sum_{28}$PCBs) and corresponding to the time interval of maximum production in Italy (Figures 2 and 3). Although low-chlorinated PCBs predominates in Ancona, the Gargano Promontory and Bari cores making up from ~ 60 to nearly 100% of the total PCBs mixture, an increase of high-chlorinated PCBs occurred during the years of maximum production in these areas of the western Adriatic Sea.

The sediments from the Po River prodelta, Ancona, the Gargano Promontory and Bari registered respective reductions of ~ 40%, ~ 50%, ~ 60%, ~ 80% in recent years, when compared to PCBs peak concentrations detected between 1960's and the 1980's. The lower concentrations found in the upper layers corresponding to the last two decades most likely reflected ban/restriction on PCB production and use in Italy due to incoming European regulations, which started to be adopted in 1976 (Tolosa et al., 1997). Despite the general decreasing trends on PCB concentrations in the past decades, their fluxes and concentrations seem to be reaching a steady state in more recent years (end of the 1990's and early 2000's), suggesting that a further reduction of PCB levels in the next years is unlikely. According to Breivik et al. (2007), PCB concentrations will return to decrease significantly after 2050, when PCBs are expected to be completely out of use. Furthermore, the whole drainage basin of the Po River would act a transient repository for PCBs, which can be slowly released over time, as occurred with transport and accumulation of radiocesium in the North Adriatic Sea following the Chernobyl accident (Frignani et al., 2004).

Although the Po River has been considered the major source of PCBs in the western Adriatic Sea, minor local inputs may also be present in Ancona and Bari areas. Actually, Ancona and Bari are urban centers sheltering two of the most important commercial and passenger harbors of the central and southern Adriatic (Mali et al., 2015), and the human activities in these coastal areas seem to be affecting PCB inputs and sediment quality. Relative higher concentrations of PCBs and other contaminants had been detected especially around the Bari port area (e.g. Guzzella and Paolis, 1994; Giandomenico et al., 2013; Mali et al., 2015).

3.5. PCB inventories

PCB inventories were assessed to estimate the total mass of PCBs in the sediment cores. Inventories can also be used to evaluate the potential of sediments as a new source of contamination to the marine ecosystem in the region. The inventories (ng cm^{-2}) were calculated as follows:

$$Inventory = \sum C_i d_i \rho_i$$

where C_i is the concentration of $\sum_{28}$PCBs in sediment layer *i* (ng g^{-1}), *d* is the thickness of the sediment layer *i* (cm) and ρ_i is the dry mass of the sediment layer *i* (g cm^{-3}; Mai et al., 2005). The PCB concentrations for unanalyzed intervals were estimated by linear interpolation of adjacent measured intervals.

The PCB inventories were estimated among the sediment layers corresponding from 1930 (first PCB production) until recent years. The inventory of PCBs was 256 ng cm^{-2} at the Po River prodelta (core J25), 63 ng cm^{-2} in Ancona (core AN2), 37 ng cm^{-2} in Bari (core BA5), 35 ng cm^{-2} at the Gargano Promontory (core GG2) and 2.5 ng cm^{-2} at the Gondola Slide (core DE15bis) (Table 1). The estimated inventory of PCB in the Po River prodelta is ~ 4 to 7 times greater in comparison to the middle and southern Adriatic, respectively, and about 100 times higher than the inventory obtained in the deep Adriatic Sea at the Gondola slide mud waves. The estimated PCBs inventories are in agreement with the detected distribution trends of total PCB in the Adriatic Sea.

PCB inventories in the Po River prodelta are higher than those detected in Venice Lagoon, Italy (32 – 80 ng cm^{-2}; Mugnai et al., 2011) and in heavily polluted tropical bays in the Philippines and in the upper Gulf of Thailand (47 - 92 ng cm^{-2}; Kwan et al.,

2014). On the other hand, PCB inventories as high as 1310 ng cm^{-2} were found in the Pearl River Delta, China, a well-known polluted site and active area for deposition of sediment-bound contaminants (Mai et al., 2005). PCB inventories in the Po River prodelta are similar to the average values reported for the top 2-cm sediments from Santa Monica Bay (USA) and for dated sediment cores from English lakes (80 ng cm^{-2} and ~ 270 ng cm^{-2}, respectively; Venkatesan et al., 2010; Yang et al., 2016).

4. Conclusions

This work provides the first extensive data set on the sources, historical patterns and inventories of PCBs in sediments from western Adriatic Sea. Total PCB concentrations, especially high-chlorinated congeners, decrease with distance from the Po River prodelta southward, suggesting the Po River outflow to be a major contributor of PCBs inputs to sediments in the western Adriatic Sea. PCB inventories, OC and C/N spatial trends corroborate this hypothesis. The occurrence of high-chlorinated PCB congeners at the Gondola slide suggests that the cascading process of the NAdDW could quickly transfer particle-bound contaminants such as PCBs from the north to the deep Adriatic Sea.

Historical trends of PCBs in the Adriatic Sea coincided with their past use and industrial production. Despite the relatively low concentrations detected, this work provides a new insight on the historical and recent PCBs contamination status in the western Adriatic Sea at a basin level. Further studies including priority and emerging contaminants are warranted to fully characterize the contamination status of the Adriatic Sea.

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Table 1

Location, depth, sediment characteristics and PCB inventories in sediment cores from the western Adriatic Sea: organic carbon (% OC), C/N atomic ratio, fine sediments (% mud), estimated SARs (g cm^{-2}), and PCB inventories (ng cm^{-2}). Minimum, maximum, mean and standard deviation (SD). Mud: silt and clay.

	Po river prodelta	Ancona	Gargano Promontory	Bari	Gondola slide
	J25	AN2	GG2	BA5	DE15bis
Latitude (N)	44°83.42'	43°42.414'	41°59.265'	41°10.611'	41°54.347'
Longitude (E)	12°58.98'	13°38.443'	16°09.066'	16°53.729'	16°58.206'
Depth (m)	26	42	34	74	522
OC	1.08 - 1.28 (1.2 ± 0.04)	0.58 - 0.83 (0.7 ± 0.05)	0.40 - 0.70 (0.6 ± 0.04)	0.6 - 0.94 (0.8 ± 0.06)	0.43 - 0.65 (0.5 ± 0.06)
C/N	9.7 - 11.7 (11 ± 0.4)	9.1 - 10.5 (10 ± 0.2)	7.1 - 10.7 (9.7 ± 0.4)	8.7 - 10.7 (9.5 ± 0.3)	7.1 - 9.6 (8.4 ± 0.7)
Mud	99.1 ± 0.35	99.2 ± 0.2	96.8 ± 0.9	97 ± 1.2	91.2 ± 1.6
Estimated SARs	0.79 ± 0.12	0.35 ± 0.04	0.46 ± 0.06	0.30 ± 0.02	0.08 ± 0.006
PCB inventories	256	63	35	37	2.5

Table 2

Depth and sediment characteristics of the surface sediments from the western Adriatic Sea: organic carbon (% OC), C/N atomic ratio, fine sediments (% mud). Mud: silt and clay.

		Depth (m)	OC	C/N	Mud
Ancona	AN1	20	0.59	8.8	94.0
	AN2	40	0.81	9.4	99.3
	AN3	65	0.91	9.2	97.6
Gargano Promontory	GG1	70	0.63	9.9	93.0
Bari	BA4	55	0.82	9.2	94.0
	BA5	78	0.83	9.4	93.8
	BA6	110	0.76	8.9	96.7
Gondola slide	DE 01	1060	0.66	8.3	88.8
	DE 06	580	0.66	8.4	94.0
	DE09	560	0.68	8.7	92.2
	DE 19	520	-	-	73.3
	DE 21	580	-	-	96.2

Table 3

PCBs ranges (ng g^{-1}, expressed at the minimum and maximum values) founded in sediments from different marine areas worldwide.

Location	PCBs (ng g^{-1})	References
Adriatic Sea	<LOD - 9.0	This work
Northern Adriatic Sea	3 - 80	Caricchia et al. 1993
Northern and Middle Adriatic	0.9 – 14.7	Fowler et al., 2000; De Lazzari et al., 2004
Western Adriatic Sea coastal areas	0.3 - 84	Pozo et al., 2009
Eastern Adriatic Sea	<0.5 - 294	Picer and Picer, 1991
Mar Piccolo, Southern Italy	2 - 1684	Cardellicchio et al., 2007
Crete, Eastern Mediterranean Sea	0.038 - 1.2	Mandalakis et al., 2014
Catalonia and Andalusia coastal areas, Mediterranean Sea, Spain	0.1 – 15.1	Eljarrat et al., 2005; Solé et al., 2013
Baltic Sea	0.71 - 74	Sobek et al., 2015
Barents Sea	<LOD – 3.5	Zaborska et al., 2011
Caribbean Sea	<LOD – 441.6	Fernandez et al., 2007 and references therein
Salton Sea, USA	116 - 304	Sapozhnikova et al., 2004
Yangtze River Estuary and East China Sea	5.08 - 19.64	Yang et al., 2012

Figure
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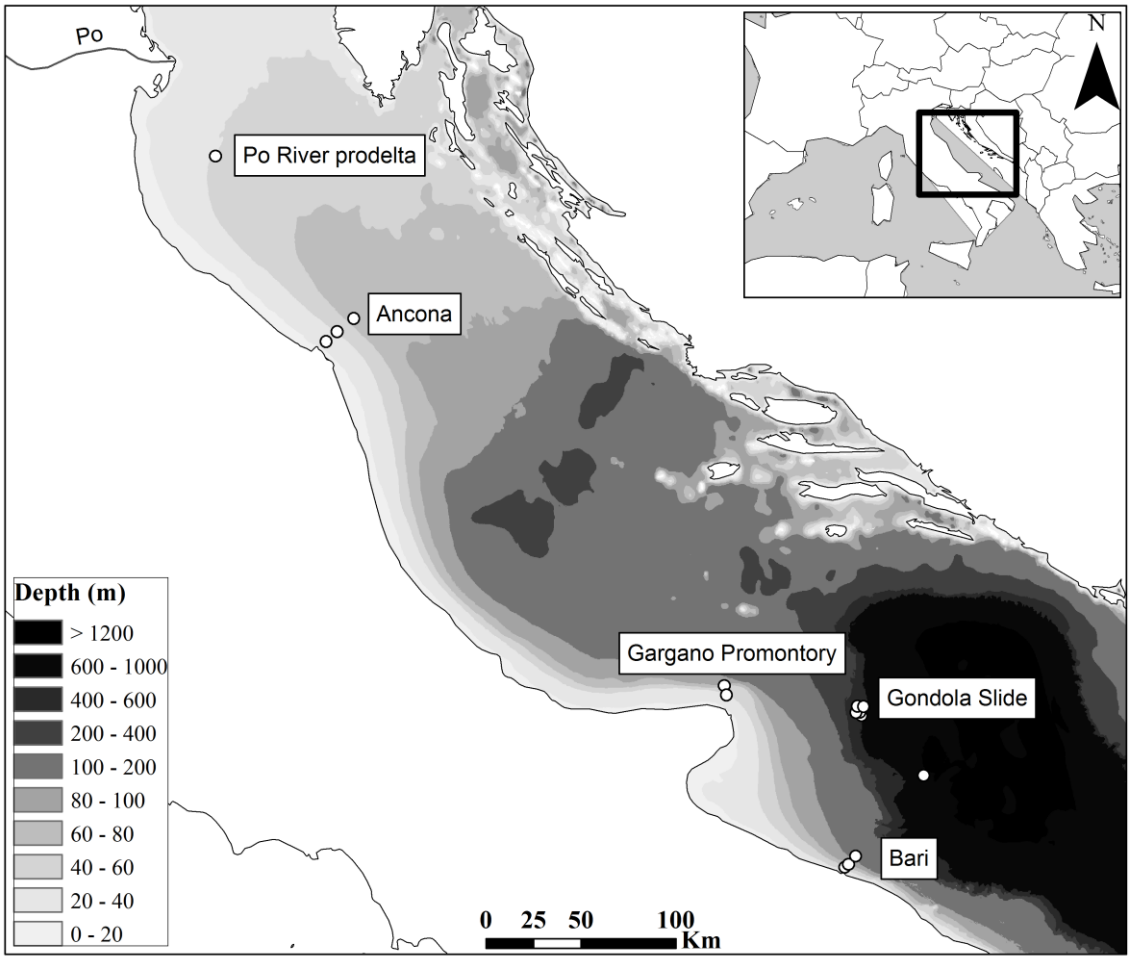

Fig. 1. Map of the Adriatic Sea indicating sediment sampling stations: the Po River prodelta, Ancona, the Gargano Promontory, Bari and the Gondola slide.

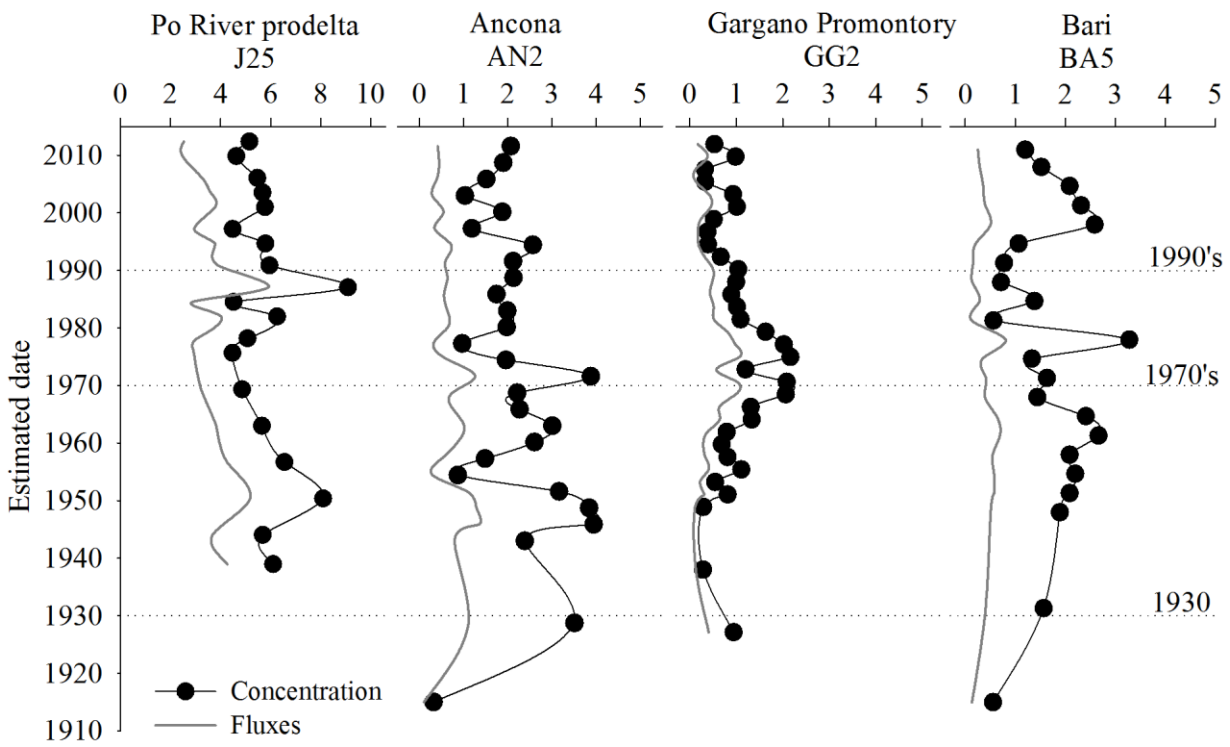

Fig. 2. Historical trends of PCBs concentrations (ng g^{-1}) and fluxes (ng cm^{-2} y^{-1}) in sediment cores from the western Adriatic Sea.

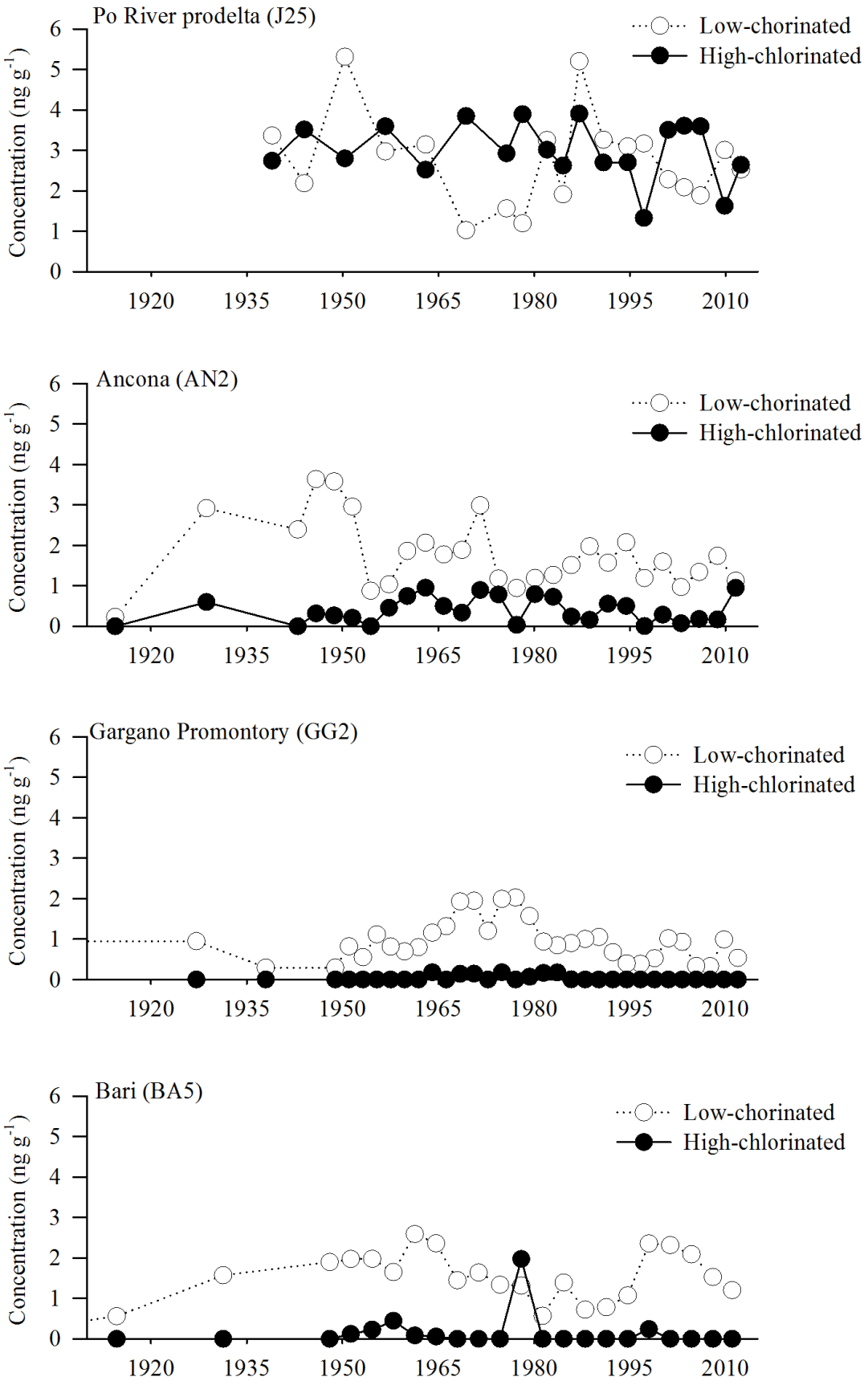

Fig. 3. Distribution of low-chlorinated (di-, tri-, tetra- and penta-CB) and high-chlorinated PCBs (from hexa- to deca-CB) in ng g^{-1} in sediment cores from the western Adriatic Sea.

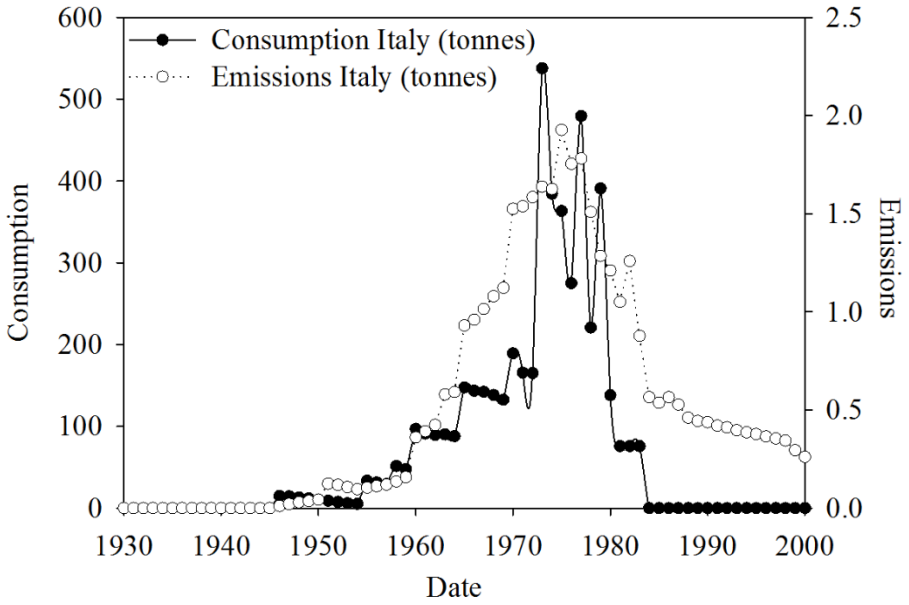

Fig. 4. Predicted temporal trends on PCB consumption and emission in Italy (according to data provided by Breivik et al., 2002a, 2007).

Supplementary material for on-line publication only

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Article II

Fate and distribution of legacy and emerging contaminants along the western Adriatic Sea

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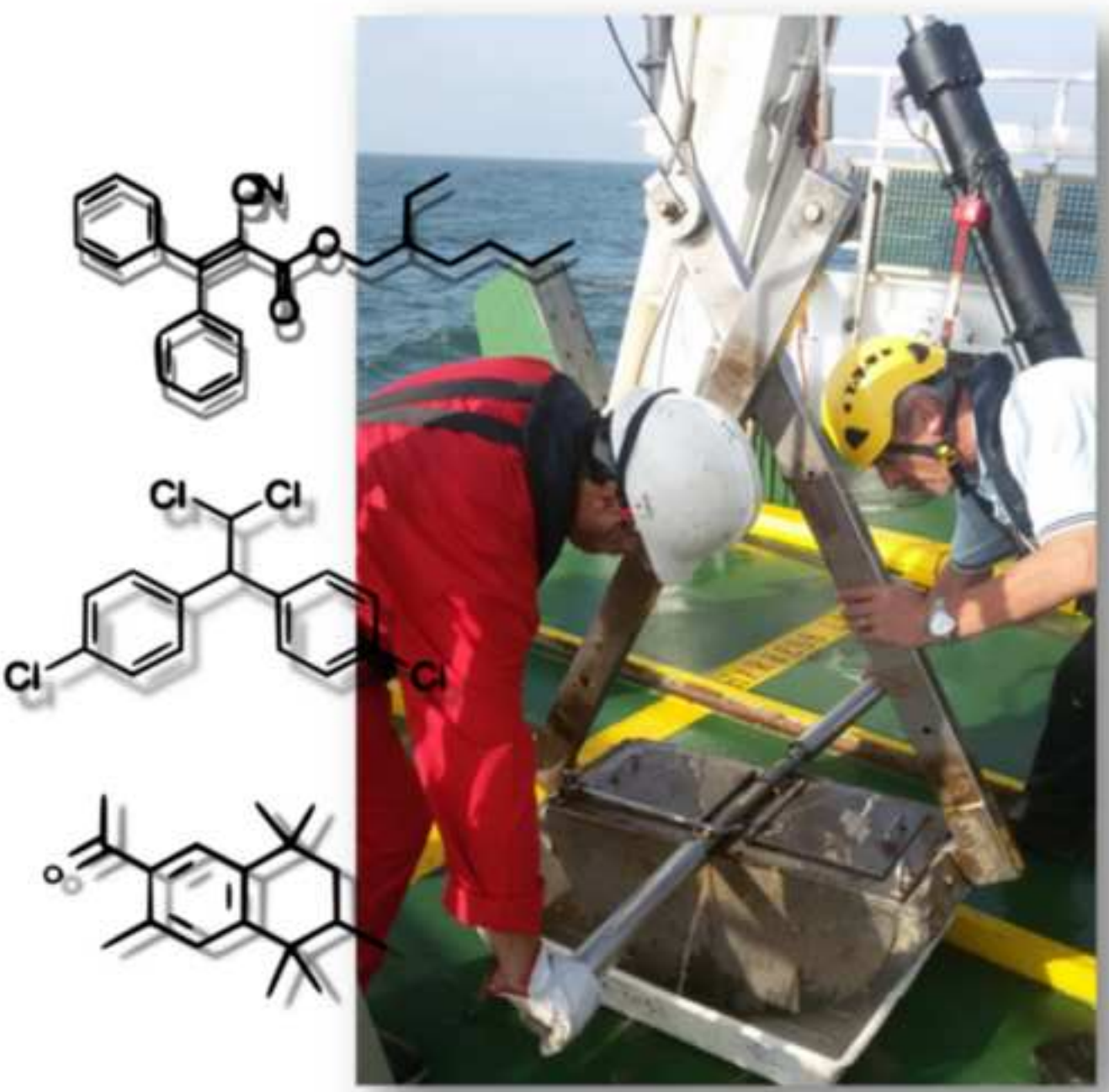
Abstract: The spatial distribution and fate of selected legacy and emerging compounds were investigated in surface sediments sampled along the Adriatic mud-wedge and in deep-sea regions from the southern Adriatic basin. Results indicated that the concentrations of legacy contaminants (PAH, PCB and DDT) and emerging contaminants (tonalide, galaxolide, EHMC, octocrylene, BP-3 and NP) ranged from 0.1 to 572 ng g-1 and from <LOD to 40.7 ng g-1, respectively. In general, higher concentrations and estimated burdens were detected in the northern Adriatic, highlighting the importance of the Po River as the major contributor for the inputs of legacy and emerging contaminants to sediments in the Adriatic Sea. Nevertheless, the prevalence of some UV filters and fragrances in the central and southern Adriatic indicates that the proximity to tourist areas and WWTPs discharges seems to affect the distribution of those compounds. The accumulation of contaminants in the deep-sea areas supports the inference that this region may act as an important repository for contaminants within the Adriatic Sea. Estimated annual contaminant accumulation reveals that both, legacy and emerging contaminants accumulate preferentially in the northern Adriatic (40 to 60% of the total annual contaminant accumulation), where the presence of legacy, and to a lesser extent emerging contaminants, are likely to pose an immediate or long-term hazard to resident biota.

Graphical Abstract



Highlights:

- Unprecedented data of contaminants levels and burden estimations were assessed
- The Po River seems to be the major contributor of legacy and emerging contaminants
- The deep Adriatic basin represents as an important repository for contaminants

Distribution and fate of legacy and emerging contaminants along the Adriatic Sea

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Abstract

The spatial distribution and fate of selected legacy and emerging compounds were investigated in surface sediments sampled along the Adriatic mud-wedge and in deep-sea regions from the southern Adriatic basin. Results indicated that the concentrations of legacy contaminants (PAH, PCB and DDT) and emerging contaminants (tonalide, galaxolide, EHMC, octocrylene, BP-3 and NP) ranged from 0.1 to 572 ng g^{-1} and from <LOD to 40.7 ng g^{-1}, respectively. In general, higher concentrations and estimated burdens were detected in the northern Adriatic, highlighting the importance of the Po River as the major contributor for the inputs of legacy and emerging contaminants to sediments in the Adriatic Sea. Nevertheless, the prevalence of some UV filters and fragrances in the central and southern Adriatic indicates that the proximity to tourist areas and WWTPs discharges seems to affect the distribution of those compounds. The accumulation of contaminants in the deep-sea areas supports the inference that this region may act as an important repository for contaminants within the Adriatic Sea. Estimated annual contaminant accumulation reveals that both, legacy and emerging contaminants accumulate preferentially in the northern Adriatic (40 to 60% of the total annual contaminant accumulation), where the presence of legacy, and to a lesser extent emerging contaminants, are likely to pose an immediate or long-term hazard to resident biota.

Keywords: emerging contaminants, Adriatic mud-wedge, hazard quotients, total mass, contaminant accumulation

Capsule

Legacy and emerging contaminants accumulate preferentially in the northern Adriatic. The NAdDW seems to transfer contaminants to deep-sea regions in the southern Adriatic.

Introduction

The Marine Strategy Framework Directive (MSFD) is a legal instrument adopted in order to achieve Good Environmental Status (GES) of the EU's marine waters by 2020 (2008/56/EC; European Commission, 2008). The MSFD is based on the use of an integrated analysis of the marine ecosystem, identifying a set of 11 descriptors of GES that aims to represent, protect and understand the functioning of the system as a whole (Borja et al., 2008; Crise et al., 2015). Among these, descriptor 8 adresses data availability, knowledge gaps and research priorities regarding contaminants and marine pollution impacts. In this sense, the recent Directive 2013/39/EU is also concerned about environmental contamination, establishing a list of 45 substances identified for priority action at Union level, including persistent organic pollutants (POPs) and contaminants of emerging concern (CECs) (European Commission, 2013; Viganò et al., 2015).

POPs (e.g. polychlorinated biphenyls - PCBs, polycyclic aromatic hidrocarbons – PAHs, and chlorinated pesticides) are a well-known group of legacy contaminants, which have been monitored and regulated in most parts of the world for the last four decades, being also referred to as “regulated contaminants” (Jones and de Voogt, 1999; Lohmann et al., 2007). On the other hand, CECs are chemical compounds that are not necessarily new, but are not or are only partly regulated and are not included in routine monitoring programs (Pintado-Herrera et al., 2016a). Despite the availability of an important amount of data sets and long time series for legacy contaminants, most of the data available refers to restricted areas and a shortage of off-shore datasets has been detected (Crise et al., 2015). Regarding CECs, although they have been increasingly studied in water, including drinking water, rivers, groundwater, wastewaters and effluents from wastewater treatment plants (WWTPs) since the 1990’s, studies focusing on the fate of emerging contaminants in the marine environment are rather scarce and the knowledge on their occurrence, fate and effects is still limited (Beretta et al., 2014; Tijani et al., 2015).

The majority of man-made substances are discharged into waterbodies, reaching coastal and marine systems. Sediments are the final repositories for most pollutants, which can accumulate and remain in the sedimentary matrix for long periods of time, from several years to decades (Ruiz-Fernández et al., 2012). Some contaminants present in sediments have the potential to accumulate through the food web, affecting marine biota, aquatic-

dependent wildlife, and ultimately human health (Borgå et al., 2001; Mourier et al., 2014). Thus, a wide-ranging work monitoring diferent groups of contaminants in sediments is a key tool for a comprehensive understanding of contaminants behavior and identification of the overall environmental quality and possible threatens to the whole marine ecosystem.

The aim of this work is to investigate levels and spatial patterns of selected groups of regulated contaminants and CEC in sediments from the Adriatic Sea (Italy), filling the gap regarding information on emerging contaminats and transfer of contaminants from coastal waters to the open sea along the Adriatic margin. This work is a part of FP7 PERSEUS (Policy-oriented marine Environmental research in the Southern European Seas), which aims to identify the interacting patterns of natural and human-derived pressures on the Mediterranean and Black Seas, linking them to the MSFD descriptors, criteria and indicators.

Study area

The Adriatic Sea is a shallow semi-enclosed basin in Southern Europe. According to Turchetto et al. (2007), the area can be divided into three sub-basins: the northern Adriatic, at the north of Ancona and with depths up to 100 m; the middle Adriatic, between Ancona and the Gargano Promontory, reaching depths until 270 m; and the southern Adriatic, from the Pelagosa Sill to the Otranto Strait, which includes the deepest area of the Adriatic Sea (the South Adriatic Pit, up to 1200m).

Freshwater input comes mainly from the Po River (northern Adriatic), which is responsible for the transport of approximately one-fourth of the material that enters the Adriatic Sea (Frignani et al., 2005). The Po delta includes five distributary mouths (the Maestra, Pila, Tolle, Gnocca and Goro), draining large agricultural and industrial areas (Romano et al., 2013; Tesi et al., 2007). The water circulation and transport of materials in the Adriatic basin is very dependent on thermohaline factors, resulting in counter-clockwise flow that varies seasonally (Artegiani et al., 1997). In general, the currents are intensified along the western Adriatic coast, exporting material southwards (Artegiani et al., 1997; Palinkas and Nittrouer, 2007). As a result of water dynamics, the suspended material accumulates in a continuous belt along the coast, forming the late-Holocene mud wedge (Frignani et al., 2005; Tesi et al., 2013). Sedimentation outside

this clinoform is negligible and not recognizable using seismic profile (Tesi et al., 2013).

During cold and dry winters, the northern Adriatic is subject to intense cooling associated with local wind forcing (Bora wind), resulting on the formation of the North Adriatic Deep Water (NAdDW), the densest water of the whole Mediterranean (Tesi et al., 2008; Vilibić and Supić, 2005). After its formation, the NAdDW spreads southwards where, because of its density, the NAdDW sinks along the bathymetric gradient (dense water cascading) reaching deep regions from the southern Adriatic basin (Langone et al., 2015; Tesi et al., 2008). The cascading of the NAdDW is responsible for the higher particle delivery in the southern Adriatic, playing a first order control on the particulate fluxes through the south-western Adriatic margin (Langone et al., 2015; Turchetto et al., 2007).

Material and methods

Sampling and sediment characteristics

In the context of the 'ADREX: Adriatic and Ionian Seas Experiment' within the PERSEUS project, a sediment sampling was performed in October 2014 on board the O/V OGS Explora in transversal-to-the-coast transects from Northern to Southern Adriatic (Figure 1). Surface sediment samples were collected along the Adriatic mud wedge, the Bari Canyon and the South Adriatic Pit by mini box corer or oceanic box corer. The top 0.5 cm of undisturbed sediment was sampled. Sediments were placed into pre-cleaned glass jars and stored at -20 °C.

Porosity (ϕ) was calculated from the loss of water between wet and dry sediment according to equations suggested by Berner (1971), assuming a sediment density of 2.65 g cm^{-3} and a water density of 1.027 g cm^{-3}. Grain size was determined after a pre-treatment with H_2O_2 and wet sieving at 63 μm to separate sands from fine fractions. Total nitrogen (TN) content were determined by elemental analysis (EA) of combusted aliquots with a Fison CHNS-O Analyzer EA 1108, and organic carbon (OC) was measured on decarbonated samples (1 M HCl).

Analytical method and instrumental analyses

The following legacy compounds were analyzed in the sediment samples: PAHs (naphthalene, acenaphthylene, acenaphthene, fluorene, anthracene, phenanthrene, pyrene, fluoranthene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene, indeno[1,2,3-cd]pyrene, benzo[g,h,i]perylene); PCBs (PCB52, PCB138, PCB153, PCB180 and PCB101) and the DDT group (*p,p′*-DDT, *p,p′*-DDD, *p,p′*-DDE). The following groups of CECs were selected for analyses: personal care products (PCPs), which included fragrances (the synthetic polycyclic musks galaxolide - HHC and tonalide - AHTN) and UV-filters (octocrylene, ethylhexyl methoxycinnamate - EHMC and benzophenone 3 -BP3), and endocrine disruptors compounds (nonylphenol isomers - NP). All are given in ng g^{-1} sediment dry weight. Further information on the suppliers for these standards and other reagents can be found in (Pintado-Herrera et al., 2016a).

First, sediments were extracted using an accelerated solvent extraction ASE 200 system (Dionex, USA) according to the extraction and in-cell clean-up method optimized by Pintado-Herrera et al. (2016a). Briefly, the extraction cells (11 mL) were prepared with 1 g of activated alumina (150°C for 16 hours; USEPA method 3610b) and 0.5 g of activated copper powder. Approximately 4 g of air-dried and milled sediment were homogenized with 1g of alumina and placed into the extraction cells. The extraction procedure consisted of three static extraction cycles using dichloromethane, where the samples were pre-heated for 5 minutes and extracted for 5 minutes in each cycle at a temperature of 100°C and a pressure of 1500 psi. The eluates were evaporated to dryness and re-dissolved in 0.5 mL of ethyl acetate. The final extracts were centrifuged (10000 rpm for 10 minutes) and filtered (0.22 µm) to remove possible interferences.

Later, separation, identification and quantification of target compounds were performed using gas chromatography (SCION 456-GC, Bruker) coupled to a triple quadrupole mass spectrometer equipped with a BR-5ms column (length: 30 m, ID: 0.25 mm, film thickness: 0.25 µm). The oven temperature was programmed to 70 ºC for 3.5 min, increasing at 25 ºC min^{-1} to 180 ºC, increasing at 10 ºC min^{-1} to 300 ºC, holding this temperature for 4 min. A derivatizing agent (*N*-(*tert*-butyldimethylsilyl)-*N*-methyltrifluoroacetamid - MTBSTFA) and internal standards (mixture of deuterated compounds) were added to the samples prior to the injection. Calibration curves were prepared for each target compound at different concentrations (from 5 to 500 ng g^{-1}).

Target compounds were identified and quantified by comparison of retention times and two transitions of each analyte (one for quantification and one for confirmation) of the samples with external standard solutions. Procedural blanks were performed for each extraction series of 10 samples using alumina and analyzed in the same way as samples. Method detection limits (MDL) were determined for each analyte as 3 times the signal to noise ratio in spiked sediment samples and were between 0.003 and 0.54 ng g^{-1} depending on the target compound. More detailed information on the methodology used can be found in Pintado-Herrera et al. (2016a).

Inventories, total burdens, contaminant accumulation and risk assessment

Inventories were calculated for surface sediment using the following equation:

$$Inventory = \sum C_i d_i \rho_i$$

where C_i is the concentration of each contaminant in sediment sample i (ng g^{-1} dry weight), d is the thickness of the sediment sampled (cm) and ρ_i is the dry mass bulk density (g cm^{-3}). In order to calculate the total burdens (mass of contaminants), the Adriatic Sea has been divided in several boxes defined by different lithological composition of the sediment source or orientation of the coastline, as suggested by Frignani et al., 2005 (Figure S1 from Supplementary Material). The total burdens were calculated by multiplying the mean calculated inventories in surface sediments by the area of the boxes (Yang et al., 2012). According to the annual sediment accumulation rate (Tg y^{-1}) estimated by Frignani et al (2005) for each box, the annual contaminant accumulation (kg y^{-1}) was also estimated.

For the preliminary risk assessment, the hazard quotients (HQs) for legacy and emerging contaminants were calculated using the measured environmental concentration (MEC) and the predicted non-effect concentration (PNEC), as follows:

$$HQ = MEC / PNEC$$

The PNEC values were either obtained from available literature or calculated using no observed effect concentrations (NOEC) from chronic toxicity bioassays or acute toxic endpoints (half maximal effective concentration, or EC50) and dividing toxicity data by a factor of 100 or 1000, respectively. Both, PNEC and calculated PNEC values, are presented in Pintado-Herrera et al. (2016b). Concentrations of target compounds in

surface sediments presented in this work were used as MEC. For interpretation, HQ < 0.1 indicates no hazard, 0.1 < HQ < 1 a low hazard, 1 < HQ >10 a moderate hazard, and HQ > 10 a high hazard (Lemly, 1996; Chen et al., 2010).

Statistical analyses

To explore the relationship between the variables, Pearson's correlation coefficient at 0.05 significance level was applied. Linear discriminant analysis was performed using the statistical package "MASS" (Venables and Ripley, 2002). Discriminant analysis is a statistical procedure for identifying boundaries between groups of samples based on quantitative predictor variables (Mourier et al., 2014). In our case, the variables used were the contaminant concentrations, and the percentage of OC and mud, while the groups were the northern, central, and southern Adriatic Sea sectors. Data were z-scoring standardized in order to eliminate the influence of different units and make each determined variable have equal weighting. Statistical data analyses were performed with R software (R Core Team, 2013).

Results

Sediment characteristics

Sediment samples were collected with preferential accumulation of fine-grained sediments along the Adriatic mud wedge and in selected deep-sea areas from the South-Western Adriatic Margin (SWAM; Cattaneo et al., 2007; Frignani et al., 2005; Romano et al., 2013; Tesi et al., 2007, 2013 and references therein). Thus, the finer fractions (<63 μm) amounted to ~50 to ~99% in analyzed sediments (Table 1). OC exhibited a relatively low content and a limited variability within the Adriatic mud-wedge sediments, varying between 0.6 and 1.6%. These levels are consistent with previous data on OC content found in the region (Tesi et al., 2013, 2007; Turchetto et al., 2007).

In general, OC and C/N ratio were higher in the northern section (1.0 ± 0.2% and 9.6 ± 0.8, respectively), especially in the samples closer to the Po River prodelta. The lowest OC and C/N values were detected in sediments off coast from central (0.5 to 0.9% and 7.6 to 9.5, respectively) and southern (0.5 to 0.8% and 7.7 to 9.4, respectively) sections.

Legacy contaminants

PAHs were by far the most prevalent legacy contaminants in surface sediments from the Adriatic Sea, with ∑16 PAHs ranging from 38.8 to 572 ng g^{-1} (Table 1). The highest concentrations (300 ±101 ng g^{-1}) were detected in the northern section, followed by the central (115.3±27.4 ng g^{-1}) and southern sections (107.4 ±64.2 ng g^{-1}). High molecular PAHs (HMW; 4–6 rings) accounted for 65 to 95% of total PAHs. The ratio between low- and high-molecular weight PAHs (LMW and HMW, respectively) ranged from 0.1 to 0.5, while the ratios between indeno[1,2,3-cd]pyrene and benzo[g,h.i]perylene (Ip/Ip+Bper), fluoranthene and pyrene (Flt/Pyr and Flt/Flt+Pyr) and benz[a]anthracene and chrysene (Ba/Ba+Chr) ranged from 0.2 to 0.6, from 0.9 to 1.5 and from 0.3 to 0.5, respectively.

The organochlorine compounds (PCBs and DDTs) were detected in relatively low concentrations and presented a similar range of concentrations. Total PCBs ($\sum_5$PCBs) and total DDTs (p,p'DDD, p,p'DDE and p,p'DDT) in surface sediments varied between 0.05 and 4.2 ng g^{-1} and between 0.05 and 4.3 ng g^{-1} respectively (Table 1). Similarly to PAHs, PCBs were also detected in higher concentrations in the northern section (2.0±0.9 ng g^{-1}) followed by the middle (0.7±0.4 ng g^{-1}) and southern (0.4±0.3 ng g^{-1}) sectors. Although total DDTs were also higher in the northern sector (1.6±1.0 ng g^{-1}), they were very similar between the central and southern sections (0.6±0.2 and 0.7±0.3 ng g^{-1}, respectively). While DDE and DDD were ubiquitous in sediments from the Adriatic Sea, DDT was detected only in 20% of the samples.

Emerging contaminants

Concentrations of fragrances ranged from <LOD (below limit of detection) to 24.3 ng g^{-1}. In general, tonalide was present in higher concentrations (6.2±4.6 ng g^{-1}, 2.9±2.2 ng g^{-1} and 6.0±2.7 ng g^{-1} in the northern, central and southern sectors, respectively) in the sediment samples in comparison to galaxolide (4.3±2.8 ng g^{-1}, 1.9±1.5 ng g^{-1} and 4.0±2.7 ng g^{-1} in the northern, central and southern sectors, respectively; Table 1). Galaxolide to tonalide ratios ranged from 0 to 5.4 (1±0.9).

UV filters ranged from <LOD to 40.7 ng g^{-1}. Octocrylene was the most abundant UV filter (16.3±9.6 ng g^{-1}, 7.6±6 ng g^{-1} and 6.8±4 ng g^{-1} in the northern, central and southern sectors, respectively), followed by EHMC (4.5±2.2 ng g^{-1}, 2.4±1 ng g^{-1} and 3.2±1.4 ng g^{-1}

267 ^{1} in the northern, central and southern sectors, respectively). Both compounds were
268 detected in all the sediment samples. Conversely, BP3 was detected at very low
269 concentrations (0.05±0.05ng g^{-1}, 0.02±0.02 ng g^{-1} and 0.06±0.06 ng g^{-1} in the northern,
270 central and southern sectors, respectively) and only in ~50% of the sediment samples.

271 NP isomers were detected in the majority of the sediment samples, ranging from <LOD
272 to 40.7 ng g^{-1}. The higher concentrations were detected in the northern sector (17±8.4
273 ng g^{-1}), while the concentrations in the central and southern sectors were very similar,
274 with mean values of 6.3±4.5 ng g^{-1} and 6.7±4.5 ng g^{-1}, respectively.

275

276 *Inventories, total burdens and contaminant accumulation*

277 PAHs ($\sum_{16}$PAHs) presented the highest inventories (mean value of 810±380 ng cm^2
278 among the three sectors), followed by octocrylene and NP (~46±19 ng cm^2). The total
279 inventories of the organochlorine compounds were very similar, with 4.8±3 ng cm^2 and
280 4.5±2 ng cm^2 for PCBs ($\sum_5$PCBs) and DDTs ($\sum$*p,p'*-DDT, *p,p'*-DDD and *p,p'*-DDE),
281 respectively. The mean inventory of the fragrances was approximately 15±4 ng cm^2 for
282 galaxolide and 22.5±5.5 ng cm^2 for tonalide. Regarding the other UV filters, EHMC
283 presented a mean inventory of 16±4.5 ng cm^2 and BP3 of 0.2±0.1 ng cm^2. The
284 estimated total mass of contaminants in the Adriatic Sea are presented in Table 1.
285 Estimated burdens in the whole Adriatic basin were nearly 15,000 kg for PAHs, 900 kg
286 for octocrylene, 765 kg for NP, 424 kg for tonalide, 330 kg for EHMC, 275 kg for
287 galaxolide, 80 kg for PCB and DDT, and 4 kg for BP3. Total annual contaminant
288 accumulation in the Adriatic Sea ranged from 0.2 for BP3 kg y^{-1} to ~7800 for PAHs kg
289 y^{-1} (Table 1). The total annual accumulations were similar for NP and octocrylene
290 (~450 kg y^{-1}) and the organochlorine compounds (~45 kg y^{-1}). EHMC, galaxolide and
291 tonalide presented similar total annual accumulation as well (~140 to 210 kg y^{-1}).

292

293 **Discussion**

294

295 *Spatial distribution of legacy and emerging contaminants*

296 PAHs, PCBs, DDTs and NP were positively correlated to OC ($r \geq 0.5$; p value ≤ 0.01),
297 suggesting that the spatial distribution of these contaminants is dependent on the OC

content of sediments. NP was strongly correlated to legacy contaminants ($r \geq 0.6$; p value < 0.001) and the UV filters (EHMC and octocrylene) were also correlated (r = 0.5; p value < 0.001), confirming these compounds present similar spatial distribution and may derive from similar input sources. The discriminant analysis explained the data variance (83.3% and 16.7% for LD1 and LD2). The scatterplot of the two discriminant functions (LD1 and LD2) shows that the north sector is better separated than the center and south (Figure 2) and PCBs, PAHs, EHMC and OC were the variables that most contributed to the group differentiation. According to the confusion matrix, the accuracy of the classification appears to be relatively high, since 70%, 80% and 90% of the samples were well reclassified within the predefined groups (central, southern and northern areas, respectively).

Although PCBs, PAHs, and EHMC were the compounds of highest importance for separating the areas, the stronger discrimination of the northern sector can be also related to the higher concentrations detected for most contaminants in this area, especially close to the Po River prodelta. On the other hand, the spatial distribution of CECs was generally not as clear as the distribution detected for legacy contaminants, especially in the central and southern Adriatic, which may explain the weak differentiation among these groups.

Spatial trends of contaminants are presented in Figures S2 to S6 of the Supplementary Material and revealed a similar pattern, with decreasing concentrations from the Po River prodelta southward to the Otranto channel and in deep areas from the South-Western Adriatic Margin (SWAM). The Po River is the largest and most important Italian river, draining large agricultural and highly industrialized areas, inhabited by 15 million of people, and being responsible for the transport of approximately one-fourth of the material that enters the Adriatic Sea (Frignani et al., 2005; Romano et al., 2013; Tesi et al., 2007). Thus, the Po River appears to be the major contributor for the inputs of legacy and emerging contaminants to sediments in the Adriatic Sea.

Although contaminants concentrations are generally lower in the central and southern sectors, some increased concentrations can be noticed especially around Ancona and Bari, which are densely anthropized areas, sheltering two of the most important commercial and passenger harbors of the Adriatic Sea (Mali et al., 2015). The human and the port-related activities in these areas may contribute to minor local inputs of contaminants. PAHs and PCBs have been previously detected in higher concentrations

in these areas, especially around the Bari port (Guzzella and Paolis, 1994; Mali et al., 2015; Combi et al., maybe). As to the fragrances, their application in a broad range of products, including washing products and detergents (OSPAR Commission, 2004), may help explaining their presence in these areas.

Fragrances and UV filters also presented somewhat higher levels in touristic coastal areas in the central and southern Adriatic, which may be related to the direct input from recreational activities (bathing, swimming). Additionally, the vicinity to major cities and touristic facilities results on an increased load of CECs from WWTPs effluents, which, in turn, represent one of their major sources to the marine environment (Chase et al., 2012; Villa et al., 2012). Previous studies also related the presence of fragrances and UV filters to both, the proximity to tourist areas and WWTPs discharges (Downs et al., 2015; Villa et al., 2012).

Both, legacy and emerging contaminants were also detected in deep sediments within the SWAM. Previous studies have suggested that the ultimate repository for contaminants are deep-sea areas located in the southern Adriatic Sea where the cascading of the North Adriatic Dense Water (NAdDW) would be able to quickly transfer suspended particles (and, therefore, particle-binding contaminants) coming from the north Adriatic (Turchetto et al., 2007; Tesi et al., 2008; Langone et al., 2015). The presence of high-chlorinated PCBs (PCB 138, PCB 153 and PCB 180), and other highly hydrophobic compounds (e.g. octocrylene and benzo[g,h,i]perylene) found in this work reinforces this hypothesis.

Comparison of the occurrence of legacy and emerging contaminants in sediments

Figure 3 illustrates the occurrence of legacy and emerging contaminants in coastal and marine areas in the southern European Seas and the Spanish Atlantic coast. Overall, the concentrations of legacy contaminants in our study were similar to those previously observed in the northern and central Adriatic Sea (De Lazzari et al., 2004). In comparison to the Po River, PAHs were detected in similar levels (Guzzella and Paolis, 1994) while DDT and PCBs presented far lower concentrations in our study ($\sum_{14}$ PCBs, Viganò et al., 2015). PAHs levels were also similar to those detected in the Black Sea (Readman et al., 2002). PCBs levels detected were lower when compared to other European coastal areas such as the Bay of Cádiz ($\sum_{5}$ PCBs, Pintado-Herrera et al.,

2016a), the Gulf of Naples ($\sum_{17}$ PCBs, Tornero and Ribera d'Alcalà, 2014) and along the eastern Mediterranean coast ($\sum_{96}$ PCBs, Barakat et al., 2013; $\sum_{28}$ PCBs, Merhaby et al., 2015) and the Black Sea ($\sum_{13}$ PCBs, Fillmann et al., 2002), but higher than levels reported in deep-sea sediments of the southern Cretan margin ($\sum_{38}$ PCBs, Mandalakis et al., 2014). DDTs were detected in comparable levels as those reported in coastal areas directly influenced by human and port activities, such as Barcelona (Tolosa et al., 1995) and Naples (Montuori et al., 2014).

Only a few studies have reported the levels of emerging contaminants in sediments, especially in transitional and marine ecosystems. The mean concentrations of the fragrances tonalide and galaxolide, the UV filter octocrylene and the endocrine disruptor NP in our study were far lower than those reported in eastern and northwestern Mediterranean coastal areas (Amine et al., 2012; Hong et al., 2009) and the Spanish Atlantic coast (Pintado-Herrera et al., 2016a). On the other hand, the mean concentrations of the UV filters BP3 and EHMC were similar to those detected in touristic areas as Cádiz Bay (Pintado-Herrera et al., 2016a) and southern France (northwestern Mediterranean coast; Amine et al., 2012).

Environmental behavior and sources of legacy contaminants

PAHs are considered ubiquitous pollutants in the marine environment and can originate from both natural processes and anthropogenic activities (Guzzella and Paolis, 1994; León et al., 2014). The incomplete combustion or pyrolysis of organic material (e.g. biomass, waste, fossil fuels) under high temperatures is one of the main sources of PAHs, as well as natural and anthropogenic petroleum spillages (Magi et al., 2002; Readman et al., 2002). The low- and high-molecular weight PAHs (LMW and HMW, respectively) ratio has been used to assess the sources of PAHs. Generally, pyrolytic sources are depleted in LMW (2–3 rings) and enriched in HMW PAHs (4–6 rings) PAHs (HMW) leading to LMW/HMW ratio < 1 (Merhaby et al., 2015), which is distinctive of the Adriatic Sea sediments. In turn, Ip/Ip+Bper, Flt/Pyr, Flt/Flt+Pyr and Ba/Ba+Chr ratios are indicative of PAHs sources from biomass and petroleum combustion (Figure 4). These ratios corroborated the pyrolytic origin of PAHs in the sediment samples from the Adriatic Sea, which is in agreement with previous research accomplished in the Adriatic Sea (Magi et al., 2002).

The most abundant PCB congeners were PCB 138 followed by PCB 180, which are the main contributors to the commercial mixtures Aroclor 1260 and Aroclor 1254 (Schulz et al., 1989). In Italy, these were the most frequently imported mixtures from the USA until the 1980s, being used mainly in electrical transformers and hydraulic fluids (Parolini et al., 2010; Pozo et al., 2009). Few studies examined PCB distributions as single congeners in sediments from the Adriatic Sea and PCB 138 was also detected in higher concentrations in a previous work (Fowler et al., 2000).

Since *p,p′*-DDE and *p,p′*-DDD are the degradation products of *p,p′*-DDT under aerobic and anaerobic conditions, respectively, the ratios of DDD+DDE/DDT can be used to evaluate whether DDT emission occurred recently or in the past (Yu et al., 2011). Our results confirm old DDT inputs in the Adriatic Sea with the prevalence of DDE, indicating that degradation occurred mainly under aerobic conditions within the top 0.5 cm of sediments. However, lower values of the DDD+DDE/DDT ratio suggesting recent DDT input were detected in a few samples, especially in the area under direct influence of the Po River prodelta. Although DDT has been banned in Italy since 1978, previous studies also pointed out to recent DDT input in diverse environmental matrixes, which was attributed to possible leaking from old chemical plants contaminated soils (Binelli and Provini, 2003; Viganò et al., 2015). Indeed, previously contaminated soils around the drainage basin of the Po River seems to be continuously contaminating waterbodies in the north of Italy, ultimately accumulating in the Adriatic Sea sediments.

Environmental behavior and sources of emerging contaminants

Galaxolide is commercially the most important polycyclic musk fragrance, followed by tonalide (Villa et al., 2012). In 2000, the production of galaxolide and tonalide in Europe was estimated on 1427 tonnes and 358 tonnes, respectively (OSPAR Commission, 2004). For this reason, galaxolide is usually detected in higher concentrations in continental, marine and transitional ecosystems, as well as in wastewaters (Chase et al., 2012; Pintado-Herrera et al., 2016a; Sumner et al., 2010). However, tonalide was found in relatively higher levels than galaxolide in Adriatic Sea, presenting galaxolide to tonalide ratios in general lower than the commercial ratio of about 4:1 (OSPAR Commission, 2004). Although both compounds present similar

physico-chemical properties (e.g., log Kow ~5.7-5.9 and vapor pressure ~0.068 – 0.073; Chase et al., 2012), previous studies suggested that galaxolide is degraded more easily than tonalide (Lee et al., 2014), and that tonalide preferentially adsorbs to particulate matter (Dsikowitzky et al., 2002), which are the most likely reasons why tonalide is ubiquitous in the Adriatic Sea sediments. Tonalide has also been detected in higher concentrations in some of the sediment samples from the Po River (Viganò et al., 2015) and Sacca di Goro Lagoon (Casatta et al., 2015).

Octocrylene was the predominant UV filter, followed by EHMC and BP3. Octocrylene is one of the most used UV filters in Europe, being present in over 80% of sunscreen products, while EHMC and BP3 can be found, respectively, in ~50% and ~20% of the products (De Groot and Roberts, 2014; Rastogi, 2002). These compounds are broadly used in cosmetics and PCPs, such as sunscreens, lip balms, shampoos and conditioners (Amine et al., 2012; Downs et al., 2015). The octanol-water partition coefficient is an indicator of the environmental fate of the UV-filters, translating how they are distributed between sediments/lipids and the aqueous phase (Ramos et al., 2015). Octocrylene is nowadays of great concern since it is a highly lipophilic compound (log K_{ow} ~7), stable, and resistant to sunlight degradation (Gago-Ferrero et al., 2013). EHMC is also a very hydrophobic compound (log K_{ow}: 5.8), while BP3 is slightly soluble in water (log K_{ow}: 3.8), making it less likely to be encountered in marine sediments.

NP isomers presented the highest concentrations among the emerging contaminants analyzed in our work. NP is an endocrine disrupting degradation product resulting from the nonionic surfactant nonylphenol ethoxylates (NPEOs) and is frequently detected in high concentrations in continental, marine and transitional waters (Pojana et al., 2007; Lara-Martín et al., 2014; Meffe and de Bustamante, 2014). Surfactants are among the most produced and consumed substances in the world and, among their degradation products, nonylphenol presents hydrophobic properties (log Ko_w: 5.7) causing a preferential accumulation in sediments (Pintado-Herrera et al., 2016a; Pojana et al., 2007). High concentrations of NP in comparison to other classes of contaminants in sediments from Venice lagoon (47 – 192 ng g^{-1}) have been atributted to the proximity to municipal and industrial wastewaters treatment plants (Pojana et al., 2007).

Fragrances, UV filters and NP can be found in relevant concentrations in both, influent and effluent wastewaters, as most WWTPs are not designed to treat these types of

substances (Chase et al., 2012; Langford et al., 2015). Because of their hydrophobic properties, the removal of emerging compounds during wastewater treatment is mainly related to their sorption on sludge solids (Carballa et al., 2004; Langford et al., 2015). For instance, the removal efficiency of NP after wastewater treatments is around 50 – 80% (Melo-Guimarães et al., 2013; Stasinakis et al., 2013), while the removal efficiency of tonalide and galaxolide can be around 85% (Carballa et al., 2004). Consequently, a relevant fraction of emerging compounds is constantly discharged through WWTPs and untreated wastewater into the aquatic environment, leading to a widespread contamination of continental, transitional and marine waters (Chase et al., 2012; Sumner et al., 2010; Villa et al., 2012). Because of their hydrophobic properties, most of these compounds are sorbed to some extent on suspended solids during wastewater treatment and as a result they can also be found in sludge.

Preliminary burden estimation, contaminant accumulation and risk assessment

Inventories and burden estimations represent the integrated mass of the compounds of interest and can be used as a tool to understand a suitable insight for further behavior of the compounds per unit area (Kim et al., 2008; Song et al., 2004). Inventories and total burdens (total mass of contaminants) were calculated with reference to the top 0.5-cm of sediment, which means that the actual inventories and total burdens would be much larger than estimated for the Adriatic Sea. Legacy contaminants presented the highest total burdens in the northern sector (40-45%) of the Adriatic Sea, while the total burdens of BP3 and the fragrances were higher in the southern sector (45-50%). Estimated burdens in the southern Adriatic are especially influenced by the higher deep-sea area in comparison to the coastal areas (Figure S1 from Supplementary Material). Total burdens in the central Adriatic ranged from 20 to 40%, with the highest values corresponding to NP and octocrylene. The similar burdens between the central and southern sectors reinforces the weak separation detected by the discriminant analysis and the presence of local sources in these areas.

Estimated annual contaminant accumulation highlights that legacy and emerging contaminants accumulate preferentially in the northern Adriatic (40 to 60% of the total annual contaminant accumulation), followed by the central (25 to 38%) and southern Adriatic (8 to 30%). Altogether ~ 12% of the legacy and emerging contaminants annually entering the Adriatic Sea accumulate in the deep Adriatic basin, which has

been previously suggested to be an important repository for sediments (Langone et al., 2015; Turchetto et al., 2007).

The annual contaminant accumulation and burden estimation are in agreement with the spatial distribution trends of legacy and emerging contaminants along the Adriatic Sea, corroborating the hypothesis that the Po River represents the major input sources of most contaminants to the Adriatic Sea.

Different approaches have been proposed for the estimation and evaluation of potential ecotoxicological risks in sediments from the Adriatic Sea. The first approach is based upon the comparison of legacy contaminants concentrations with environmental quality standards (EQS) for sediments of marine, coastal and transitional environments, as set by the Italian Decree n. 260/2010 (D.M. 260/2010) in application of the MSFD. The northern sector of the Adriatic Sea displayed the highest concentrations of contaminants exceeding the EQS for benzo[k]fluoranthene (EQS: 20 ng g^{-1}), benzo[b]fluoranthene (EQS: 40 ng g^{-1}), indeno[1,2,3]pyrene (EQS: 70 ng g^{-1}) in 56%, 25%, and 6% of the sediments, respectively, and for *p,p′*-DDD (EQS: 0.8 ng g^{-1}) and *p,p′*-DDE (EQS: 1.8 ng g^{-1}) in 25% and 12.5% of the sediments, respectively. *p,p′*-DDE was above the EQS in 15% of the sediments from the southern Adriatic, while no sediments from the central section exceeded the national EQS values.

For the second approach, we calculated the hazard quotients (HQs) for individual legacy and emerging contaminants. Emerging contaminants present no significant ecological risk in sediments of the Adriatic Sea except for the UV filter EHMC, which poses moderate risk for sediment-associated biota. The HQs for individual compounds suggested a high risk of adverse effects to biota related to total PAHs, especially phenanthrene, pyrene, benzo[a]anthracene, benzo[a]pyrene, and dibenzo[a,h]anthracene, *p,p′*-DDE and PCBs in the northern sector, while only dibenzo[a,h]anthracene and *p,p′*-DDE represent a high ecotoxicological risk for organisms in the central and southern sectors.

Environmental matrices contaminated with diverse groups of pollutants are complex in terms of understanding the interaction mechanisms among different compounds; previous studies have demonstrated that the presence of many chemicals may have addictive toxicological effect (Cristale et al., 2013). In order to investigate the overall risk of contaminants in sediments from the Adriatic Sea, individual HQs were combined and divided by the number of HQs, similarly to the approach proposed by Long et al.

(2006) for the assessment of mean Sediment Quality Guidelines (SQGs). The combined HQs (~3 and ~4, respectively) for central and southern Adriatic Sea suggest a moderate hazard for sediment-associated biota, while in the northern Adriatic section combined HQ suggests high ecotoxicological hazard (HQ = ~10). Along with the fact that a considerable portion of the samples in this area also exceeds the national EQSs for several legacy compounds, we can infer that legacy, and to a lesser extent emerging contaminants present in sediments from the northern Adriatic Sea are likely to pose an immediate or long-term hazard to resident biota.

Conclusions

The occurrence of emerging and legacy contaminants was investigated in surface sediments along the Adriatic mud wedge and in selected deep-sea areas from the South-Western Adriatic Margin (SWAM). To the best of our knowledge, this is the first study on CEC occurrence, levels and distribution in sediments along the western Adriatic Sea. Spatial trends of legacy and emerging contaminants revealed a similar pattern, with decreasing concentrations from the Po River prodelta southward, suggesting the Po River as the major inputs contributor to sediments in the Adriatic Sea. This inference is further corroborated by the distribution patterns for OC and annual contaminant accumulation along the Adriatic Sea, with higher values consistently detected in the northern section. A significant presence of emerging compounds has been detected in the southern Adriatic, especially fragrances and UV filters, most likely related to diffuse sources as touristic activities and WWTPs discharges.

The hypothesis that the deep-sea areas in the southern Adriatic may represent the final repository for contaminants entering this system has been reinforced by the annual contaminant accumulation estimated for this area. The transfer of contaminants from coastal waters to the open sea has been related to the cascading of the North Adriatic Dense Water (NAdDW) in deep-sea areas in the southern Adriatic, which would be able to quickly transfer suspended particles (and, therefore, particle-binding contaminants).

Concentrations exceeded the national EQSs for sediments for individual PAHs, DDD and DDE, especially in the northern Adriatic, where the combined HQs for legacy and emerging contaminants suggested a significant ecotoxicological hazard, whereas the central and southern sections seem to be at moderate risk of adverse ecological effects.

Thus, the ecotoxicological risk to sediment-associated biota should not be neglected, particularly in the northern Adriatic. Further studies are warranted to fully characterize the ecological risk in sediments with a particular attention to the northern Adriatic Sea.

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Figure
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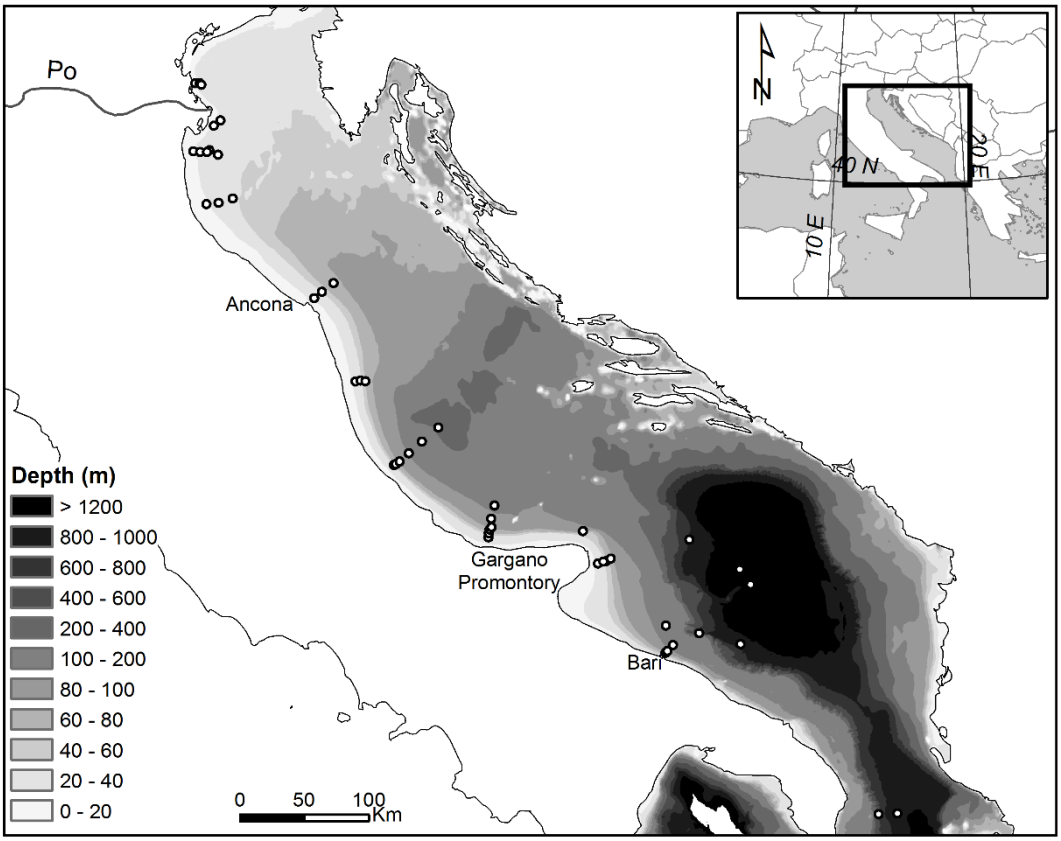

Figure 1. Map of the study area showing the sampling stations and the bathymetry of the Adriatic Sea.

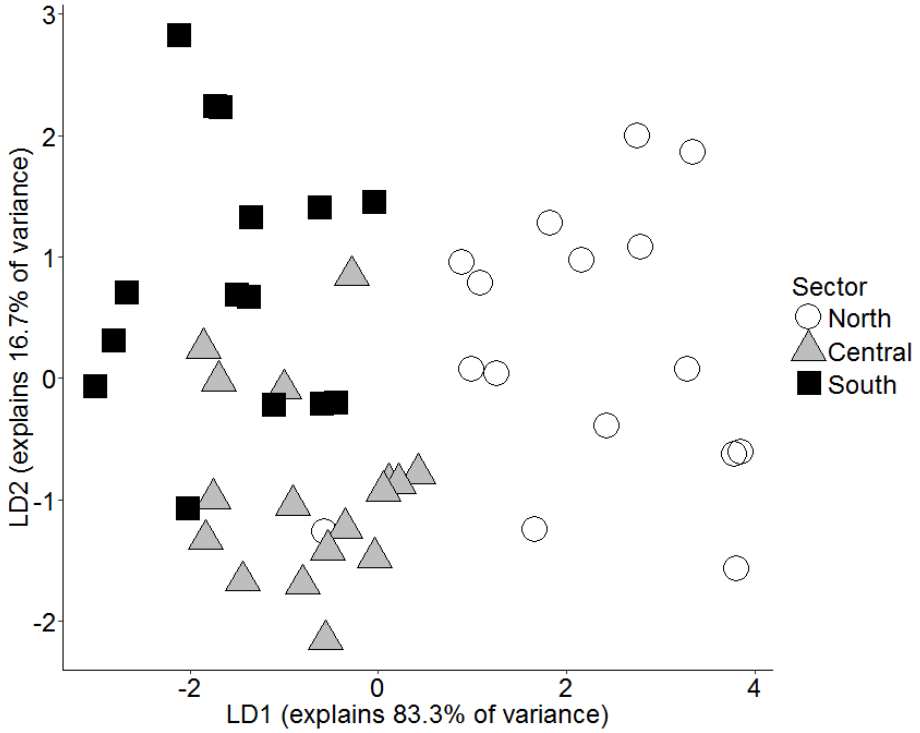

Figure 2. Discriminant analysis scatterplot on legacy and emerging compounds in the northern, central and southern sections of the Adriatic Sea.

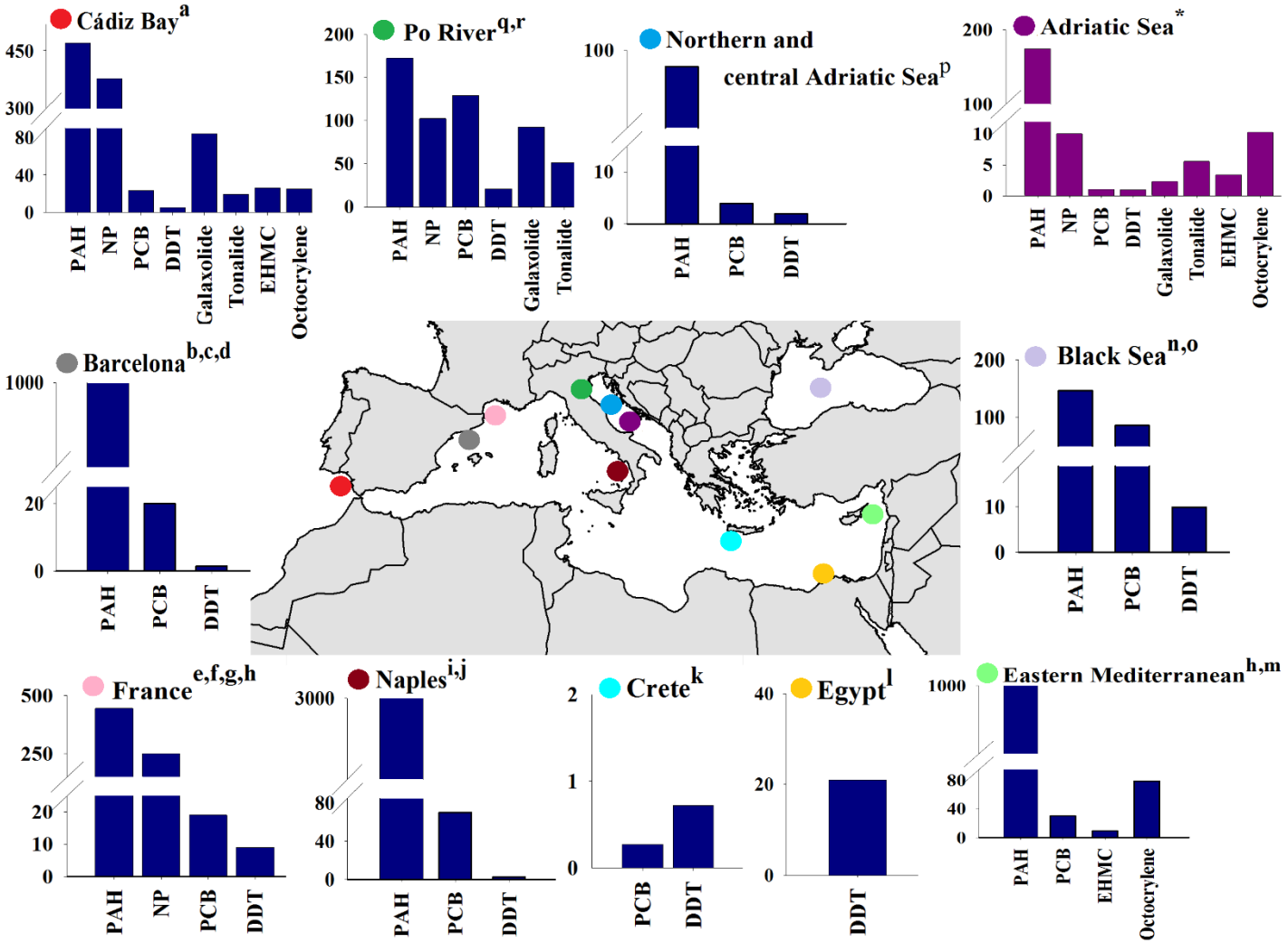

Figure 3. Comparison of legacy and emerging contaminants concentrations (mean value, ng g^{-1}) in sediments from the southern European Seas and coastal areas and the Spanish Atlantic coast. *: Present study; [a]Pintado-Herrera et al. (2016); [b]Castells et al. (2008); [c]León et al. (2014); [d]Tolosa et al. (1995); [e]Salvadó et al. (2013); [f]Bouloubassi et al (2012); [g]Hong et al. (2009); [h]Amine et al. (2012);[i]Tornero and Ribera d'Alcalà (2014); [j]Montuori et al. (2014); [k]Mandalakis et al. (2014); [l]Barakat et al. (2013); [m]Merhaby et al. (2015); [n]Fillmann et al. (2002); [o]Readman et al. (2002); [p]De Lazzari et al. (2004);[q]Viganó et al. (2015); [r]Guzzella and Paolis (1994).

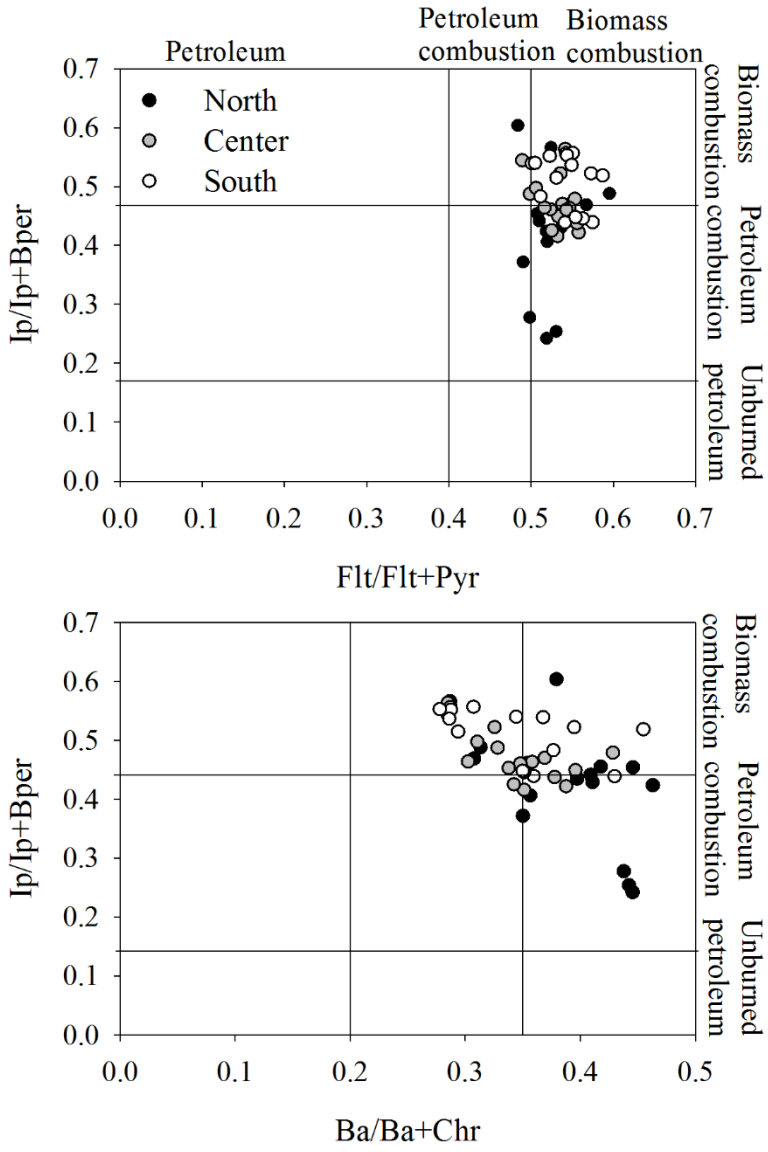

Figure 4. Cross plots of Ip/Ip+Bper versus Flt/Flt+Pyr and Ip/Ip+Bper versus Ba/Ba+Chr for the sediment samples in the northern, central and southern Adriatic Sea.

Table
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Table 1. Sediment chemical data summaries for the Adriatic Sea. Data show the mean, standard deviation, minimum, maximum and 50th (i.e. median value) and 95th percentile for n= 48 samples (ng g^{-1}), the estimated burdens of contaminants (kg) and the annual contaminant accumulation (kg y^{-1}) for each area (northern, central and southern Adriatic). Mud (silt and clay) and OC are presented as %.

		Range		Summary statistics				Burdens	Accumulation
		Min	Max	Mean	SD	50th	95th		
Northern Adriatic	Mud	94.0	99.5	98.0	1.0	98.5	99.5	-	-
	OC	0.6	1.6	1.0	0.2	0.9	1.3	-	-
	C/N	8.0	12.5	9.6	0.8	9.2	12.0	-	-
	PAH	103.6	572	300	101.0	300	500	5400	4330
	PCB	0.3	4.3	2.0	0.9	1.9	3.7	35	30
	DDT	0.2	4.3	1.6	1.0	1.2	4.2	32	25
	Tonalide	1.3	24.3	6.2	4.6	3.9	19.0	85	85
	Galaxolide	0.05	11.9	4.3	2.8	3.6	10.7	50	58
	Octocrylene	4.0	40.7	16.3	9.6	12.3	35.0	260	230
	BP3	<LOD	0.23	0.05	0.05	0.03	0.18	1.5	0.8
	EHMC	1.0	10.4	4.5	2.2	3.8	9.8	115	68
	NP	3.2	40.7	17.0	8.4	15.2	38.0	265	235
Central Adriatic	Mud	89.0	99.6	98.3	1.5	99.2	99.6	-	-
	OC	0.5	0.9	0.7	0.1	0.8	0.9	-	-
	C/N	7.6	9.5	8.5	0.4	8.3	9.3	-	-
	PAH	62.0	166.0	115.3	27.4	120.8	165.0	4800	2640
	PCB	0.1	1.2	0.7	0.4	0.9	1.2	28	16
	DDT	0.1	1.0	0.6	0.2	0.7	1.0	23	13
	Tonalide	0.9	13.6	3.0	2.2	1.5	8.2	128	70
	Galaxolide	<LOD	7.3	1.9	1.5	1.4	7.0	87	46
	Octocrylene	0.8	33.7	7.6	6.0	5.0	20.6	370	187
	BP3	<LOD	0.1	0.02	0.02	0.00	0.08	1	1
	EHMC	0.9	6.9	2.4	1.0	2.2	4.5	104	56
	NP	<LOD	19.2	6.3	4.5	5.3	19.0	298	153
Southern Adriatic	Mud	52	99.6	91.8	9.0	99.7	99.4	-	-
	OC	0.5	0.8	0.7	0.1	0.7	0.7	-	-
	C/N	7.7	9.4	8.4	0.5	8.3	9.3	-	-
	PAH	38.8	368.5	107.4	64.2	72.0	252.2	3230	890
	PCB	<LOD	0.8	0.4	0.3	0.4	0.8	14	4
	DDT	0.3	1.5	0.7	0.3	0.6	1.3	24	7
	Tonalide	0.6	13.4	6.0	2.7	5.3	11.8	210	56
	Galaxolide	0.2	16.0	4.0	2.7	2.5	12.3	138	38
	Octocrylene	0.9	19.0	6.8	4.0	5.7	18.4	265	70
	BP3	<LOD	0.18	0.06	0.06	0.05	0.15	2	1
	EHMC	1.3	10.0	3.2	1.4	1.7	7.0	108	30
	NP	0.5	31.8	6.7	4.5	4.8	16.6	200	55

Supplementary Material

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Article III

Sedimentary record of PCBs, DDTs and PAHs in coastal and deep-sea sediments from the Adriatic Sea (Italy)

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Persistent organic pollutants (POPs) are among the most concerning groups of legacy contaminants due to well-known characteristics as high persistence in the environment, hydrophobicity and toxicity (Lohmann et al., 2007). Thus, regulatory efforts and international agreements have been made in past decades in order to reduce future environmental burdens of POPs (Breivik et al., 2004; Vallack et al., 1998). Internationally, POPs are regulated Under the Stockholm Convention under which the signatory countries are legally required to eliminate the production, use, and emissions of POPs, with the ultimate goal of reducing human and ecosystem exposure (Holoubek and Klánová, 2008; Nizzetto et al., 2010). Parallel efforts have been made at European level; for instance the recent Directive 2013/39/EU established a list of 45 substances identified for priority action at Union level, including some POPs as polychlorinated biphenyls (PCBs) and dichlorodiphenyl-trichloroethanes (DDTs), and some polycyclic aromatic hydrocarbons (PAH) (European Commission, 2013; Viganò et al., 2015).

The regulatory actions have led to the reduction or elimination of major primary sources associated with the production and use of POPs, followed by a gradual decline on environmental levels of these contaminants over time, especially after the 1980's (Combi et al., 2013; Franců et al., 2009; Smith et al., 2009). However, there are still ongoing primary releases from diffuse sources that are difficult to target for reduction or elimination, such as volatilization from old stockpiles, or from old equipment that is still in use (Breivik et al., 2002; Nizzetto et al., 2010). Additionally, these compounds are highly persistent in the environment and continue to be found in different environmental matrixes and their current levels are not expected to decrease significantly within the next decades (Breivik et al., 2007; Sobek et al., 2015). Therefore, it is essential to understand the contaminant loadings, both that we may assess the effects of environmental legislation and that we may identify emerging sources of pollution (Kannan et al., 2005).

Coastal and marine areas are the final receptacle for human waste and contaminants, where sediments usually act as a sink for most pollutants (Argiriadis et al., 2014; Sahu et al., 2009). Due to their specific physical-chemical characteristics (e.g. octanol-water partition coefficient and chemical stability), PCBs, DDTs and PAHs can accumulate and persist in the sedimentary matrix for long periods of time, from years to several decades (Ruiz-Fernández et al., 2012). Consequently, sediment cores can represent a record of historical inputs and trends of contaminants, being a helpful instrument to

understand the overall environmental quality of marine systems (Hong et al., 2003; Martins et al., 2010; Santschi et al., 2001).

The aim of this work is to reconstruct the historical inputs and estimate the deposition fluxes of selected classes of PCBs, DDTs and PAHs in sediment cores from: (a) a coastal area nearby a highly industrialized region in the north of Italy (Po River prodelta); and (b) a deep-sea area at the South-Western Adriatic Margin (SWAM). This work is part of the PERSEUS EU FP7 Project (Policy-oriented marine Environmental research in the Southern European Seas), which presents as one of the main goals the understanding of the contaminants transfer of contaminants from coastal areas to open sea sediments along the western Adriatic margin.

In the context of the 'ADREX: Adriatic and Ionian Seas Experiment' within the PERSEUS project, a sediment sampling was performed in October 2014 on board the O/V OGS Explora at the Po River prodelta (station 9) and in deep-sea areas at the South-Western Adriatic Margin (SWAM; stations 1 and 2; Figure 1). Sediment cores (length $\leq$ 50 cm; diameter: 10 cm) were collected using a cylindrical box-corer or the gravity sediment corer SW104, specially designed to preserve the sediment-water interface, and sectioned onboard at 1-cm intervals. Sediments were placed into pre-cleaned glass jars and stored at -20 °C until processing and analysis.

Grain size was determined after a pre-treatment with H_2O_2 and wet sieving at 63 μm to separate sands from fine fractions. Total carbon (TC), nitrogen (TN) content were determined by elemental analysis (EA) of combusted aliquots with a Fison CHNS-O Analyzer EA 1108, and organic carbon (OC) was measured on decarbonated samples (1 M HCl). Stable isotopic analyses of organic C ($\delta^{13}C$) were carried out on the same samples using a FINNIGAN Delta Plus mass spectrometer directly coupled to the FISONS NA2000 EA by means of a CONFLO interface for continuous flow measurements.

Since information on accumulation rates and strata chronologies in the deep Adriatic is scarce, sediment cores sampled in the South Adriatic (station 1 and station 2) were measured for ^{210}Pb activities to obtain information on sediment accumulation rates (SARs) and strata chronologies. The estimated date for the sediment core from the Po River prodelta (station 9) was assessed according to the extensive dataset available on sediment accumulation rates based on radioisotope geochronology (mainly 210Pb and

137Cs) in the Adriatic Sea (Frignani et al., 2005; Palinkas and Nittrouer, 2007; Tesi et al., 2013). SARs were 0.105 cm y-1 at Station 1, 0.103 cm y-1 at Station 2 and 0.52 cm y-1 at Station 9.

In order to estimate the date for each section of the sediment cores, the sediment accumulation rate reported for each key-station was used, as follows:

$$\text{Estimated date [}\textit{anno Domini}\text{ (A.D.)]} = a - \left(\frac{b}{c}\right)$$

where a is the year in which the core was collected, b is the depth of the section in the core and c is the SAR of each core.

Annual fluxes of contaminants (ng cm^{-2} y^{-1}) were estimated as follows:

$$\text{Flux} = C_i r \rho_i$$

where C_i is the concentration of $\sum_{28}$PCBs in sediment layer i (ng g^{-1}), r is SAR in the sediment core (cm y^{-1}) and ρ_i is the dry mass bulk density of the sediment layer i (g cm^{-3}).

The following compounds were analyzed in the sediment samples: PAHs (naphthalene, acenaphthylene, acenaphthene, fluorene, anthracene, phenanthrene, pyrene, fluoranthene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene, indeno[1,2,3-cd]pyrene, benzo[g,h,i]perylene); PCBs (PCB52, PCB138, PCB153, PCB180 and PCB101) and the DDT group (*p,p′*-DDT, *p,p′*-DDD, *p,p′*-DDE). Further information on the suppliers for these standards and other reagents can be found in (Pintado-Herrera et al., 2016a).

First, sediments were extracted using an accelerated solvent extraction ASE 200 system (Dionex, USA) according to the extraction and in-cell clean-up method optimized by Pintado-Herrera et al. (2016a). Briefly, the extraction cells (11 mL) were prepared with 1 g of activated alumina (150°C for 16 hours; USEPA method 3610b) and 0.5 g of activated copper powder. Approximately 4 g of air-dried and milled sediment were homogenized with 1g of alumina and placed into the extraction cells. The extraction procedure consisted of three static extraction cycles using dichloromethane, where the samples were pre-heated for 5 minutes and extracted for 5 minutes in each cycle at a temperature of 100°C and a pressure of 1500 psi. The eluates were evaporated to

dryness and re-dissolved in 0.5 mL of ethyl acetate. The final extracts were centrifuged (10000 rpm for 10 minutes) and filtered (0.22 μm) to remove possible interferences.

Later, separation, identification and quantification of target compounds were performed using gas chromatography (SCION 456-GC, Bruker) coupled to a triple quadrupole mass spectrometer equipped with a BR-5ms column (length: 30 m, ID: 0.25 mm, film thickness: 0.25 μm). The oven temperature was programmed to 70 ºC for 3.5 min, increasing at 25 ºC min^{-1} to 180 ºC, increasing at 10 ºC min^{-1} to 300 ºC, holding this temperature for 4 min. A derivatizing agent (*N*-(*tert*-butyldimethylsilyl)-*N*-methyltrifluoroacetamid - MTBSTFA) and internal standards (mixture of deuterated compounds) were added to the samples prior to the injection. Calibration curves were prepared for each target compound at different concentrations (from 5 to 500 ng g^{-1}). Target compounds were identified and quantified by comparison of retention times and two transitions of each analyte (one for quantification and one for confirmation) of the samples with external standard solutions. Procedural blanks were performed for each extraction series of 10 samples using alumina and analyzed in the same way as samples. Method detection limits (MDL) were determined for each analyte as 3 times the signal to noise ratio in spiked sediment samples and were between 0.003 and 0.54 ng g^{-1} depending on the target compound. Detailed information on the methodology can be find in Pintado-Herrera et al. (2016a).

PAHs were the prevalent contaminants in the sediment cores from the Adriatic Sea. The concentrations of $\sum_{16}$PAHs ranged from 193 to 533 ng g^{-1} (mean 363 ± 59 ng g^{-1}), from 24 to 74 ng g^{-1} (mean 48.3 ± 16 ng g^{-1}), and from 11 to 49 ng g^{-1} (mean 25 ± 9 ng g^{-1}) in the sediment cores from the Po River prodelta (station 9) and from the deep southern Adriatic margin (stations 1 and 2), respectively. The incomplete combustion or pyrolysis of organic material (e.g. biomass, waste, fossil fuels) under high temperatures is one of the main sources of PAHs, as well as natural and anthropogenic petroleum spillages (Magi et al., 2002; Readman et al., 2002). The low- and high-molecular weight PAHs (LMW and HMW, respectively) ratio has been used to assess the sources of PAHs. Generally, pyrolytic sources are depleted in LMW (2–3 rings) and enriched in HMW PAHs (4–6 rings) PAHs (HMW) leading to LMW/HMW ratio < 1 (Merhaby et al., 2015).

HMW accounted for 75 to 90% of total PAHs among the three sediment cores and the ratio between low- and high-molecular weight PAHs (LMW and HMW, respectively)

ranged from 0.1 to 1.4, indicating pyrolytic sources. In turn, the ratios between indeno[1,2,3-cd]pyrene and benzo[g,h.i]perylene (Ip/Ip+Bper), fluoranthene and pyrene (Flt/Pyr and Flt/Flt+Pyr) and benz[a]anthracene and chrysene (Ba/Ba+Chr) ranged from 0.4 to 0.6, from 0.3 to 1.2 and from 0.1 to 0.4, respectively (Figure 2). These rates indicate PAHs sources from petroleum combustion in the Po River prodelta (station 9) and from biomass combustion in the southern Adriatic (stations 1 and 2), corroborating the pyrolytic origin of PAHs in the sediment samples from the Adriatic Sea and also in agreement with previous research (Magi et al., 2002).

Total PCBs ($\sum_5$PCBs) varied between 0.9 to 5.2 ng g^{-1} (mean 2.4 ± 0.8 ng g^{-1}), between 0.1 to 2.1 ng g^{-1} (mean 0.5 ± 0.3 ng g^{-1}) and between <DL to 1.4ng g^{-1} (mean 0.3 ± 0.3 ng g^{-1}) in sediment cores from station 9, station 1 and station 2, respectively (Figures 3 and 4). PCB concentrations in stations 1 and 2 were very low and close to the limit of detection, and only two congeners were detected (PCB 138 and PCB 180). Those congeners were predominant in station 9, accounting for 45% and 20% of total PCBs, respectively. PCB 138 and PCB 180 are the main contributors to the commercial mixtures Aroclor 1260 and Aroclor 1254 (Schulz et al., 1989), which were the most frequently imported mixtures in Italy until the 1980s, being used mainly in electrical transformers and hydraulic fluids (Parolini et al., 2010; Pozo et al., 2009). Few studies examined PCB distributions as single congeners in sediments from the Adriatic Sea and PCB 138 was also detected in higher concentrations in a previous work (Fowler et al., 2000).

Concentrations of ∑DDTs (*p,p′*-DDT, *p,p′*-DDD, *p,p′*-DDE) ranged from <DL to 2.5 ng g^{-1} (mean 1.0 ± 0.4 ng g^{-1}) in the Po River prodelta (station 9) and from <DL to 2.1 ng g^{-1} (mean 0.6 ± 0.6 ng g^{-1}) and <DL to 0.7 ng g^{-1} (mean 0.3 ± 0.3 ng g^{-1}) in the southern Adriatic (stations 1 and 2, respectively). Since *p,p′*-DDE and *p,p′*-DDD are the degradation products of *p,p′*-DDT under aerobic and anaerobic conditions, respectively, the ratios of DDD+DDE/DDT can be used to evaluate whether DDT emission occurred recently or in the past (Yu et al., 2011). Our results confirm old DDT inputs in the Adriatic Sea, since *p,p′*-DDT has not been detected in the majority of the samples. The metabolite *p,p′*-DDE was predominant, corresponding to around 70% of total DDTs and suggesting that degradation occurred mainly under aerobic conditions.

Calculated contaminants fluxes followed the same patterns as those detected for total concentrations (data not shown). Organochlorine compounds concentrations were non-

detectable or negligible in the southern Adriatic (stations 1 and 2), which make those sediment cores unsuitable for the reconstruction of historical record. In turn, PAHs concentrations were rather constant in this area, with a slight increase from the lower horizons to the middle sections of sediment cores from the southern Adriatic, corresponding to the period after the 1960's. Similarly, contaminants concentrations also showed an increasing trend in the sediment core from the Po River prodelta (station 9) from the 1960's onwards, until the 1980's. The Po River has a drainage basin of 75,000 km^2 being one of the main drainage basins in Europe and has been considered the major source of contaminants in the western Adriatic Sea. The influence of the Po River discharge can be noted in the sediment core from station 9, where increasing concentrations were detected subsequently to major flood events.

PAHs, PCBs and DDTs presented a first peak concentration at the beginning of the 1950's, corresponding to a major Po River's flood occurred in November 1951 (Figure 4). According to data obtained from the Italian Regional Agency for Environmental Protection and Control (ARPA), a mean discharge close to 8,000 m^3s^{-1} was registered during this heavy flood event. Although concentrations started to decrease after the 1980's, a slight increase on PAHs, PCBs and DDTs concentrations can be identified after the Po River's flood from November 1994, when a mean discharge of 9,000 m^3s^{-1} has been registered. This influence is clearly confirmed by the decreasing trend for $\delta^{13}C$, indicating the predominance of terrestrial sources, within the same period. Large floods can mobilize upstream contaminants sources, resulting in inputs of contaminated sediments, and thus affect pollutants distribution (Mourier et al., 2014).

200 **References**

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Figures

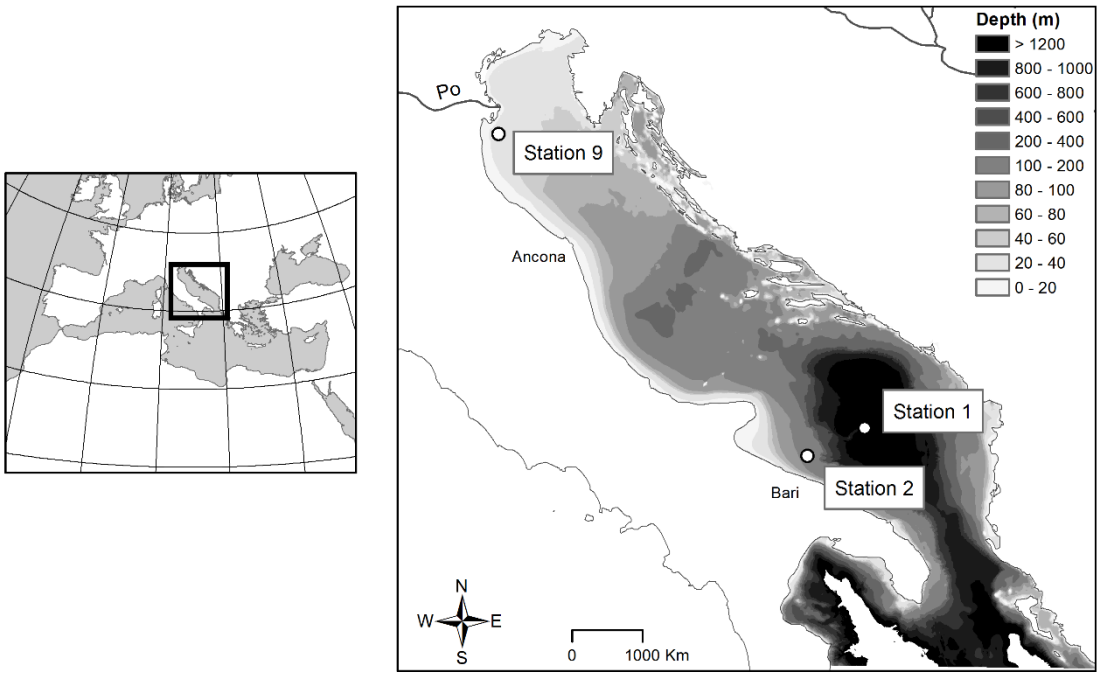
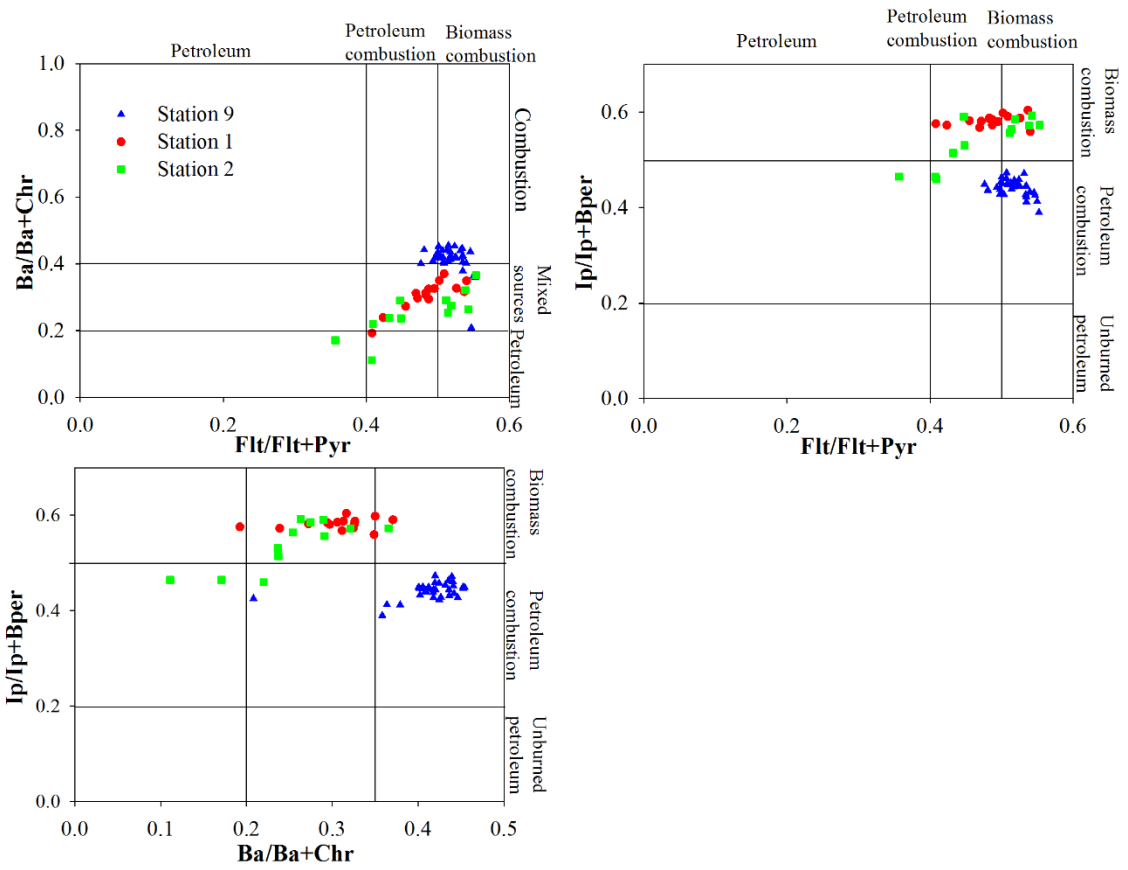


Figure 1. Map of the study area showing the sampling stations and the bathymetry of the Adriatic Sea



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314 **Figure 2.** Cross plots of Ba/Ba+Chr and Ip/Ip+Bper versus Flt/Flt+Pyr and Ip/Ip+Bper
315 versus Ba/Ba+Chr for the sediment cores from the Po River prodelta (station 9) and
316 southern Adriatic (stations 1 and 2).

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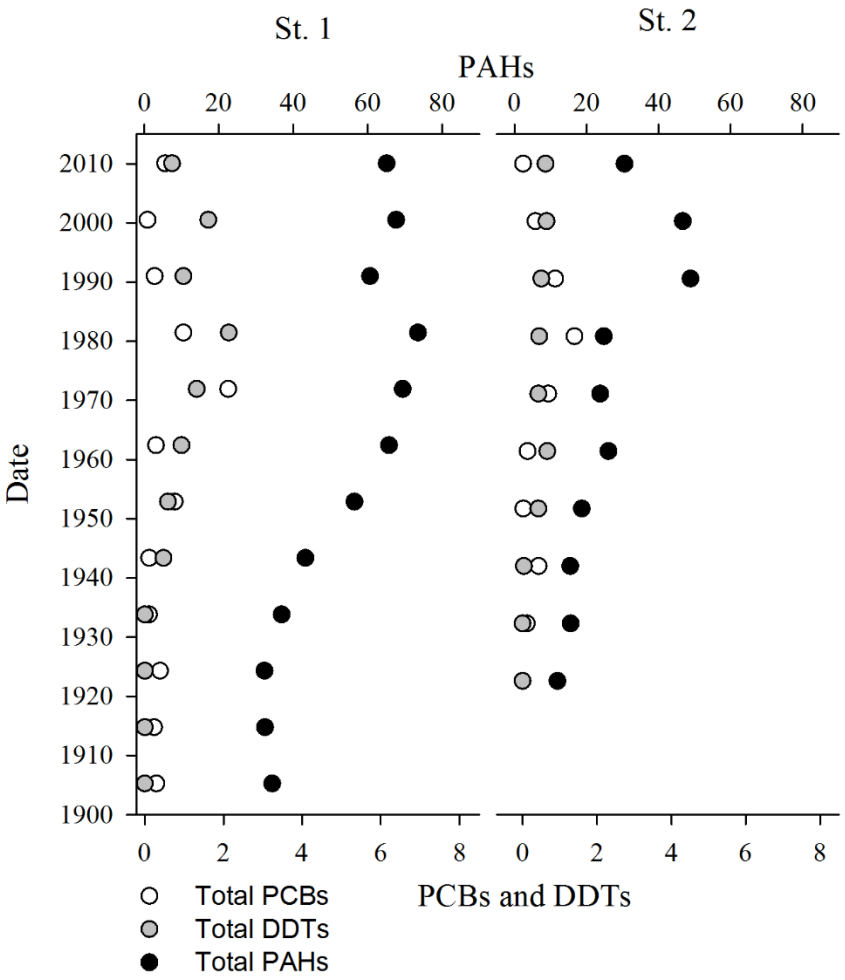
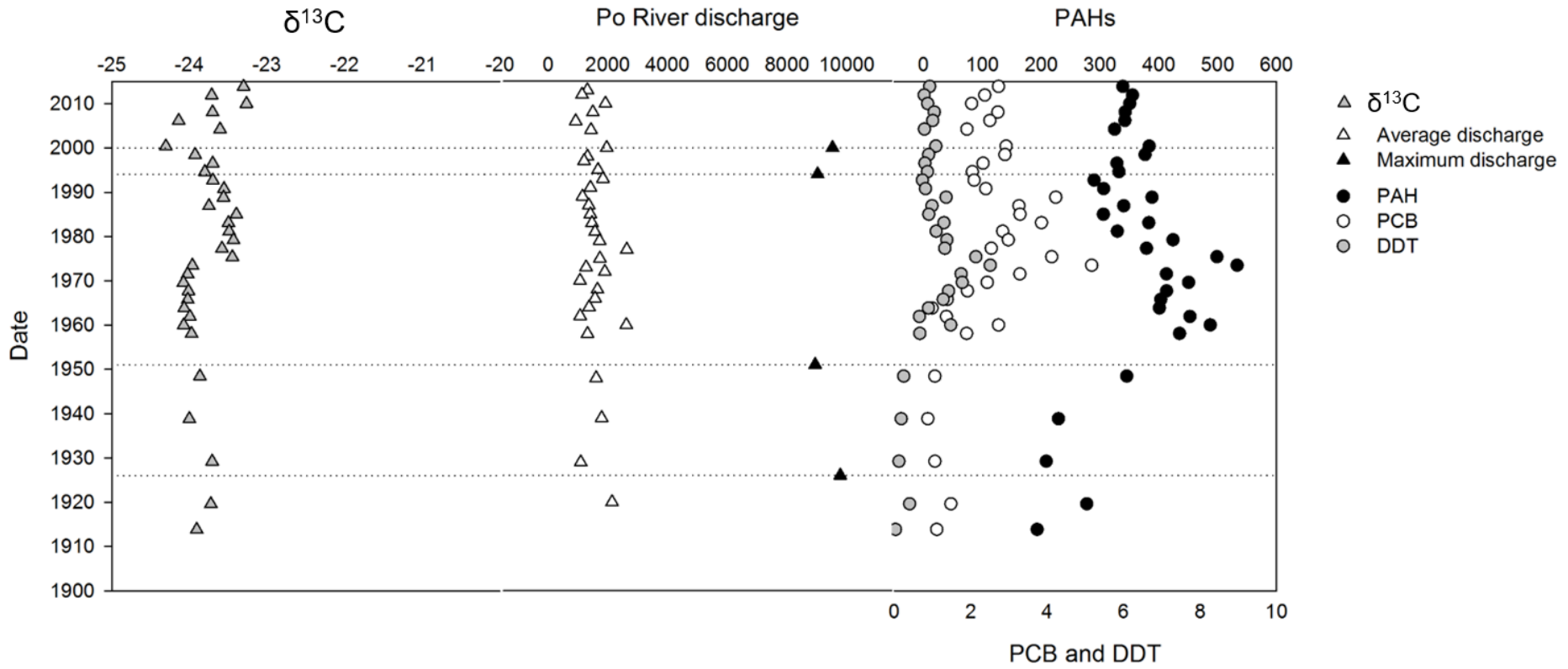


Figure 3. Vertical profile of PAHs, DDTs and PCBs concentrations (ng g^{-1}) in sediment cores from the southern Adriatic Sea (stations 1 and 2).



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322 **Figure 4.** Vertical profile of PAHs, DDTs and PCBs concentrations (ng g^{-1}), $\delta^{13}C$ variation (‰) in sediment cores from the southern Adriatic Sea (stations

323 1 and 2) and Po River's mean annual discharge and mean daily discharges during flood events (m^3s^{-1}).

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CHAPTER 4

Conclusions

This work provides the first extensive data set on the levels and contamination status of several groups of legacy and emerging contaminants in sediments from the Adriatic Sea. These results provide significant information about sources, environmental concentrations and distribution of pollutants in the region, filling important gaps indicated on MSFD descriptor 8 ("Concentrations of contaminants are at levels not giving rise to pollution effects") regarding the current knowledge and data availability on legacy and emerging compounds. The results presented in this doctoral thesis also successfully met one of the main aims in the context of the 'ADREX: Adriatic and Ionian Seas Experiment' within the FP7 PERSEUS program (Policy-oriented marine Environmental research in the Southern European Seas), which is related to the north-south transfer of pollutants along the western Adriatic Sea. Briefly, the main conclusions related to this work were:

(1) The maximum concentrations of legacy compounds (PCBs, DDTs and PAHs) were detected in the Po River Prodelta area and the lowest concentrations close to Otranto channel. Spatial trens of these contaminants revealed a common pattern, with decreasing concentrations from the Po River prodelta southward, suggesting the Po River outflow to be a major contributor of these contaminants inputs to sediments in the western Adriatic Sea. Contaminants inventories, OC and C/N spatial trends corroborated this hypothesis.

(2) Diverse groups of contaminants of emerging concern (CEC) were analyzed for the first time in sediments along the western Adriatic Sea, including personal care products (PCPs), such as fragrances (tonalide and galaxolide) and UV-filters (octocrylene, benzophenone-3 and EHMC), and endocrine disruptors (e.g. nonylphenol). Although CECs also presented higher concentration in the Northern Adriatic, the spatial distribution was not as clear as the one detected for the regulated compounds. Fragrances and UV filters presented some

increased concentrations close to turistic areas as the Gargano Promontory, Ancona and Bari, besides the Po River Prodelta area.

(3) Concentrations for some contaminants exceeded the national environmental quality standards (EQSs), especially in the northern Adriatic, where the estimated hazard quotient (HQs) for legacy and emerging contaminants suggested a significant ecotoxicological hazard, whereas the central and southern sections seem to be at moderate risk of adverse ecological effects. Thus, the ecotoxicological risk to sediment-associated biota should not be neglected, particularly in the northern Adriatic. Further studies are warranted to better assess the ecological risk in sediments from the Adriatic Sea.

(4) Altogether, the results of this work reinforced the hypothesis that the deep-sea areas in the southern Adriatic may represent the final repository for contaminants entering thisbasin. The transfer of contaminants from coastal waters to the open sea has been related to the cascading of the North Adriatic Dense Water (NAdDW) in deep-sea areas in the southern Adriatic, which would be able to quickly transfer suspended particles, and therefore, particle-binding contaminants. Further studies focused on monitoring of inputs and deposition over time in water, suspended material and marine biota are warranted to fully characterize the source, transfer and accumulation of legacy and emerging contaminants within the Adriatic Sea basin.

APPENDICES

APPENDIX A: Scientific outreach and dissemination of results

1. Seminars and congresses

- Guerra, R.; Combi, T.; Nuzzo, A.; Zanaroli, G.; Fava, F. (2013) Changes in metal partitioning during anaerobic biodegradation of polychlorinated biphenyls in co-contaminated sediments. **ECOMONDO 2013**, Rimini, Italy *(extended abstract and poster presentation)*
- Combi T., Guerra R. (2014). Extraction of PCBs in spiked marine sediments using accelerated-solvent extraction (ASE) and Soxhlet extraction. **ECOMONDO 2014**, Rimini, Italy *(extended abstract and poster presentation)*.
- Combi, T., Martins, C.C., Taniguchi, S., Leonel, J., Lourenço, A.R., Montone, R.C. (2014). Vertical distribution of Polychlorinated Biphenyls (PCBs) in sediment cores from Admiralty Bay, King George Island, Antarctica. **XXXIII SCAR 2014**, Open Science Conference, Auckland, New Zealand *(abstract and poster presentation)*.
- Zoppini A, Ademollo N., Amalfitano S., Combi T., Dellisanti W., Miserocchi S., Patrolecco L., Guerra R., Langone L. (2014). Organic priority substances and microbial processes in marine coastal sediments (Adriatic Sea, Italy). **PERSEUS 2nd Scientific Workshop**, Marrakesh, Morocco *(poster presentation and abstract).*
- Combi, T., Miserocchi, S., Langone, L., Guerra, R. (2015). Historical sediment record and distribution of polychlorinated biphenyls (PCBs) in sediments from the Adriatic Sea (Italy). **SETAC Europe 25th Annual Meeting**, Barcelona, Spain *(poster presentation and abstract).*
- Combi, T., Taniguchi, S., Martins, C.C. (2015). Depositional history of polychlorinated biphenyls (PCBs) in two South Atlantic subtropical estuarine systems. **SETAC Europe 25th Annual Meeting**, Barcelona, Spain *(poster presentation and abstract).*
- Combi, T., Martins, C.C., Taniguchi, S., Leonel, J., Lourenço, R.A., Montone, R.C. (2015). Sources and of Polychlorinated Biphenyls (PCBs) in Admiralty Bay, King

George Island, Antarctica. **SETAC Europe 25th Annual Meeting**, Barcelona, Spain *(poster presentation and abstract)*.

- Machado, A., Combi, T. (2015). Assessment of pollution and environmental restoration in coastal areas: challenges and solutions. **YOUMARES 6**, Bremen, Germany *(review).*
- Correa, A.C., Dauner, A.L.L., Combi, T., Martins, C.C., (2015). Hydrocarbons concentrations in sediments of Guaratuba bay, SW Atlantic, Brazil. **XVI Congreso Latinoamericano de Ciencias del Mar (COLACMAR)**, Santa Marta, Colombia *(video cartel and abstract).*
- Combi, T., Langone, L., Miserocchi, S., Lara-Martín, P.A., Pintado-Herrera, M.G., Guerra, R. (2016) Spatial distribution of legacy and emerging contaminants in sediments from the Western Adriatic Sea (Italy). **SETAC Europe 26th Annual Meeting**, Nantes, France *(poster presentation and abstract)*. Accepted.
- Rocha, M.L., Combi, T., Langone, L., Miserocchi, S., Guerra, R. (2016) Spatial patterns of target metals (Cu, Pb, Zn) in recent sediments from the Adriatic Sea. **SETAC Europe 26th Annual Meeting**, Nantes, France *(poster presentation and abstract)*. Accepted.
- Le Thanh, K.B., Do Hong, L.C., Werner, I., Combi, T., Lara-Martin, P.A., Casado-Martínez, M.C. (2016) Sediment quality assessment in Ho Chi Minh City canals. **SETAC Europe 26th Annual Meeting**, Nantes, France *(poster presentation and abstract)*. Accepted.

2. Articles

- Combi, T., Miserocchi, S., Langone, L., Guerra, R. Polychlorinated biphenyls (PCBs) in sediments from the western Adriatic Sea: sources, historical trends and inventories. Journal: **Science of the Total Environment** *(revised version submitted)*
- Combi, C., Pintado-Herrera, M.G., Lara-Martín, P.A., Miserocchi, S., Langone, L., Guerra, R. Fate and distribution of legacy and emerging contaminants along the western Adriatic Sea. Journal: **Environmental Pollution** *(submitted)*
- Combi, C., Pintado-Herrera, M.G., Lara-Martín, P.A., Miserocchi, S., Langone, L., Guerra, R. Sedimentary record of PCBs, DDTs and PAHs in coastal and deep-sea

sediments from the Adriatic Sea (Italy). Journal: **Marine Pollution Bulletin** *(in preparation)*

- Pintado-Herrera, M.G., Combi, T., González-Mazo, E., Lara-Martín, P.A. Occurrence and spatiotemporal distribution of legacy and emerging organic pollutants in coastal marine sediments from the Atlantic coast (Andalusia, SW Spain) *(in preparation)*
- Combi, T., Martins, C.C., Taniguchi, S., Leonel, J., Lourenço, A.R., Montone, R.C. Depositional history and sources of polychlorinated biphenyls (PCBs) in sediment cores from an Antarctic Specially Managed Area (Admiralty Bay, King George Island). Journal: **Chemosphere** *(under review).*

3. Oceanographic cruises

- **ADRI 13**

  The oceanographic cruise was performed on November 2013 on board the O/V Dallaporta under the coordination of the Consiglio Nazionale delle Ricerche - Istituto di Scienze Marine (CNR-ISMAR). Sediment samples were collected in transversal-to-the-coast transects from Ancona to Bari, Italy (Adriatic Sea).

- **ADREX 14**

  The oceanographic cruise was performed on October 2014 on board the O/V OGS Explora under the coordination of the Istituto Nazionale di Oceanografia e di Geofisica Sperimentale (OGS, Triste, Italy). Sediment samples were collected in transversal-to-the-coast transects from Trieste to Otranto, Italy (Adriatic Sea).

4. Research abroad and in other institutions

- Consiglio Nazionale delle Ricerche, Istituto di Scienze Marine – CNR/ISMAR (November 2014 – February 2015). Analyzes of sediment characteristics (e.g. grain size, organic matter content) in sediment samples from the western Adriatic Sea under the supervision of Dr. Stefano Miserocchi and Dr. Leonardo Langone.

- Departamento de Química Física, Facultad de Ciencias del Mar y Ambientales, Universidad de Cádiz, Spain (March – October 2015). Analyzes of emerging and legacy contaminants in sediment samples from the western Adriatic Sea under the supervision of Prof. Pablo Antonio Lara-Martín.

5. Teaching activities

- A.A. 2013-2014. Prevenzione e Controllo dell'Impatto Ambientale. Corso di Laurea Magistrale in Analisi e Gestione dell'Ambiente (Alma Mater Studiorum – Università di Bologna). Seminar: Class on Persistent Organic Pollutants (2 h).
- A.A. 2014-2015. Prevenzione e Controllo dell'Impatto Ambientale. Corso di Laurea Magistrale in Analisi e Gestione dell'Ambiente (Alma Mater Studiorum – Università di Bologna). Seminar: Class on Persistent Organic Pollutants (4 h).
- A.A. Course 2015-2016. Prevenzione e Controllo dell'Impatto Ambientale. Corso di Laurea Magistrale in Analisi e Gestione dell'Ambiente (Alma Mater Studiorum – Università di Bologna). Seminar: Class on Persistent Organic Pollutants and Emerging Contaminants (4 h).
- A.A. 2013-2014. B. Paganeli. M.S. student, Universidade Federal de São Carlos, Brazil; Analysis of polychlorinated biphenyls (PCBs) in sediments from the Adriatic Sea. Recipient of Programa Ciência sem Fronteiras Brazil fellowship – Internship M.Sc. Marine Biology, Alma Mater Studiorum – Università di Bologna (co-advisor).

APPENDIX B: Sediment sampling for pollutant distribution and fluxes in the Adriatic Sea

Sediment sampling for pollutant distribution and fluxes in the Adriatic Sea

Leonardo Langone[1], Tatiane Combi[2], Marilia Lopes Da Rocha[2]

[1]CNR-ISMAR, UOS Bologna, [2]CIRSA, University of Bologna in Ravenna

Introduction

In the general frame of PERSEUS, the contribution CNR-ISMAR in the ADREX experiment is to provide data useful to define natural and human-derived pressures and their impacts on deep marine ecosystems of the southern Adriatic sea.

CNR-ISMAR proposes to better characterize origin, transfer, pathways and fate of pollutants (organic metals, metals, etc.) in the Southern Adriatic Sea, in strict connection with activities planned in WP2 in the Northern Adriatic Sea. In particular, CNR-ISMAR is mainly interested to investigate the distribution of pollutants in the Po river dispersion system from the prodelta area to the Southern Adriatic, by means of surface sediments and a number of selected undisturbed sediment cores on which absolute age is measured, allowing the determination of the complete record of the temporal contamination history. This makes possible to better define natural background concentrations and present levels of pollution for trace metal (with special focus on Pb, Zn and Hg) and contaminants (with special focus on PCB and DDT).

Rationale

In the Adriatic Sea, the dispersion of riverborne materials is driven by the general cyclonic water-circulation system and by the oceanographic conditions. As a consequence, fine sediments accumulate in a belt that parallels the Italian coast. River prodeltas are the areas of first deposition of suspended particles supplied by rivers. The combined effects of waves, currents and biomixing are able to resuspend sediment particles from temporary

deposits and disperse them along or across the continental shelves as far as their final repositories. Furthermore, many of the processes that drive the behavior of the marine environment are mediated by particulate material, such as those driving the cycle of organic carbon and the fate of anthropogenic chemicals. Therefore, the quantitative assessment of mechanisms and patterns of pollutant transport and accumulation can provide basic information on both natural and anthropogenic processes, useful for defining the good environmental status. The sediment mass balance of the western Adriatic sea has established that only ~10% total riverine sediment supply escapes the accumulation on the continental shelf and reaches the South Adriatic basin or is exported out the Otranto strait (Frignani et al., 2005). The cascading of NAdDW through the Bari canyon was demonstrated to enhance transfer of sediment toward the deep basin (Turchetto et al., 2007), whilst data of pollutants were never measured during a cascading event.

In the last century, the southern Adriatic has been repeatedly used as dump site for unexploded weapons, whereas at what extent land-derived pollutants are accumulated in the Southern Adriatic basin is completely unknown.

Planned CNR-ISMAR products

- Mass balances of specific metals and organic contaminants will be calculated for the Adriatic Sea based on contents of pollutants and mass accumulation rates by ^{210}Pb, ^{137}Cs and/or ^{14}C.
- Historical reconstruction of pollution by inventories and contents of contaminants in selected sediment cores along the Adriatic continental shelf, slope and deep basin, and at the Otranto southernmost boundary.

The sampling plan

To gain information not only on pollutant amounts accumulating in the sediments of the Adriatic, but also which are the pollutant sources it was decided to cover the entire area from Trieste to Otranto. It was decided to carry out a number of transects on which to perform the sampling of surface sediments and a selection of short sediment cores.

The sampling plan was drawn up based on detailed morpho-bathymetric maps (Trincardi et al., 2013) and taking into account the average sedimentation rates for the last century from

reference data (Frignani et al., 2005, Palinkas and Nittrouer, 2006, 2007; Alvisi, 2009). All the chosen sites have a known sediment accumulation rate. This will allow us to calculate the flux of burial of each measured parameter (contaminants or what else). Furthermore, it was planned to collect short sediment cores along the fluvial dispersion system that will allow us the historical reconstruction of pollution of all parameters taken into account and compare it with their input function. In particular, significant levels to be considered will be the age of the onset, the maximum concentration level, the possible occurrence of an upward decreasing trend, the calculation of the restoration time if the polluting input stopped in a certain time, and so on, and how they vary along the dispersal system of the Po river.

The sediment sampling was carried out for multiple teams including the research groups of Annamaria Zoppini (IRSA-CNR, Rome) and Michele Giani (OGS, Trieste).

Equipment used to collect sediment

- 60 liter grab
- Oceanic Box corer (about 800 kg)
- Mini box corer (about 100 kg)
- SW104 sediment corer for undisturbed sediment sampling

Activities carried out on board R/V OGS Explora

- Sampling of surface sediment
- Sampling of short sediment cores
- Subsampling of the short sediment cores in slices 1-2 cm thick.

In the table below the list of stations occupied together with the type of collected sample (surface sediment or sediment core), the subsampling and the planned use.

Station	Instrumentation	Subsampling	Date	Time (UTC)	Latitude GGMM.xxx	Longitude GGMM.xxx	Corr. water depth (m)	Planned analysis*	Comments
66	GRAB	Surface	11/10/2014	04:45	4522.4837	1227.5265	16	OC, OM, grain size, Hg, porosity	
15	GRAB	Surface	11/10/2014	06:42	4508.9872	1221.4563	17	OC, OM, grain size, Hg, porosity	
16	GRAB	Surface	11/10/2014	08:00	4509.0101	1224.0442	22	OC, OM, grain size, Hg, porosity	
10	GRAB	Surface	11/10/2014	08:13	4508.3975	1225.0505	23	OC, OM, grain size, Hg, porosity	
8	SW104-CORER 1	Short core	12/10/2014	*FAILED*	*FAILED*	FAILED		*FAILED*	
9	SW104-CORER 2	Short core	13/10/2014	*FAILED*	*FAILED*	FAILED		*FAILED*	
10	SW104-CORER 3	Short core	11/10/2014	09:42	4508.3809	1225.0809	23	Microbiology	
10	SW104-CORER 4	Short core	11/10/2014	10:33	4508.3925	1225.0416	23	OC, OM, grain size, Hg, porosity	33 cm long (sliced @ 1 cm)
10	SW104-CORER 5	Short core	11/10/2014	11:14	4508.3887	1225.0443	23	x-ray, magnetic susceptibility	47 cm long (whole)
65	GRAB	Surface	11/10/2014	12:53	4458.1160	1237.4193	29	OC, OM, grain size, Hg, porosity	
64	GRAB	Surface	11/10/2014	13:40	4453.6685	1237.0420	24	OC, OM, grain size, Hg, porosity	
63	GRAB	*FAILED*	11/10/2014	*FAILED*	*FAILED*	FAILED		*FAILED*	*GRAB did not work*
62	MINI BOX CORER	Surface	11/10/2014	16:30	4451.3953	1233.2167	20	OC, OM, grain size, Hg, porosity	

Station	Instrumentation	Subsampling	Date	Time (UTC)	Latitude GGMM.xxx	Longitude GGMM.xxx	Corr. water depth (m)	Planned analysis*	Comments
61	GRAB	Surface	11/10/2014	17:06	4450.0438	1235.3902	25	OC, OM, grain size, Hg, porosity	
17	GRAB	Surface	12/10/2014	04:25	4440.3927	1221.5608	15	OC, OM, grain size, Hg, porosity	
18	GRAB	Surface	12/10/2014	05:28	4440.1738	1225.6260	20	OC, OM, grain size, Hg, porosity	
9	GRAB	Surface	12/10/2014	09:44	4441.0070	1231.0370	26	OC, OM, grain size, Hg, porosity	
9	SW104-CORER 1	Short core	12/10/2014	10:27	4441.0188	1231.0582	26	Microbiology	
9	SW104-CORER 2	*FAILED*	12/10/2014	*FAILED*	*FAILED*	FAILED		*FAILED*	
9	SW104-CORER 3	Short core	12/10/2014	10:57	4441.0135	1231.0710	27	OC, OM, grain size, Hg, porosity	50 cm long (sliced @ 1 cm)
9	SW104-CORER 4	Short core	12/10/2014	11:53	4441.0065	1231.0703	26	x-ray, magnetic susceptibility	46.5 cm long (whole)
19	MINI BOX CORER	Surface	12/10/2014	13:05	4440.2967	1229.6625	25	OC, OM, grain size, Hg, porosity	
20	MINI BOX CORER	Surface	12/10/2014	14:15	4439.2203	1236.1585	30	OC, OM, grain size, Hg, porosity	
25	GRAB	Surface	12/10/2014	16:44	4420.9870	1245.5462	35	OC, OM, grain size, Hg, porosity	
24	GRAB	Surface	12/10/2014	17:49	4418.9995	1237.3527	24	OC, OM, grain size, Hg, porosity	

Station	Instrumentation	Subsampling	Date	Time (UTC)	Latitude GGMM.xxx	Longitude GGMM.xxx	Corr. water depth (m)	Planned analysis*	Comments
23	GRAB	Surface	12/10/2014	18:27	4418.4933	1233.8810	19	OC, OM, grain size, Hg, porosity	
22-15	GRAB	Surface	12/10/2014	19:08	4418.3132	1230.2453	22	OC, OM, grain size, Hg, porosity	
26	GRAB	Surface	13/10/2014	07:53	4339.4842	1334.1260	20	Microbiology, Hg	
8	GRAB	Surface	13/10/2014	08:45	4342.3780	1338.3673	42	Microbiology, Hg	
8	SW104-CORER 1	Short core	13/10/2014	09:12	4342.3322	1338.3285	42	Microbiology, Hg	50 cm long (sliced @ 2 cm)
27	GRAB	Surface	13/10/2014	10:37	4346.1395	1344.7887	67	Microbiology, Hg	
58	GRAB	Surface	13/10/2014	15:16	4305.1140	1358.7452	21	OC, OM, grain size, Hg, porosity	
59	GRAB	Surface	13/10/2014	15:46	4305.5213	1401.6177	33	OC, OM, grain size, Hg, porosity	
60	GRAB	Surface	13/10/2014	16:13	4305.2140	1404.4395	54	OC, OM, grain size, Hg, porosity	
28	GRAB	Surface	14/10/2014	06:11	4228.6342	1419.2760	22	OC, OM, grain size, Hg, porosity	
29	GRAB	Surface	14/10/2014	06:43	4230.0942	1421.2505	44	OC, OM, grain size, Hg, porosity	
30	GRAB	Surface	14/10/2014	07:04	4230.4648	1422.2312	59	OC, OM, grain size, Hg, porosity	
7	GRAB	Surface	14/10/2014	07:22	4230.7937	1422.9067	65	OC, OM, grain size, Hg, porosity	

Appendix B

Station	Instrumentation	Subsampling	Date	Time (UTC)	Latitude GGMM.xxx	Longitude GGMM.xxx	Corr. water depth (m)	Planned analysis*	Comments
7	SW104-CORER 1	Short core	14/10/2014	07:35	4230.7878	1422.8970	65	OC, OM, grain size, Hg, porosity	48 cm long (sliced @ 1 cm)
7	SW104-CORER 2	Short core	14/10/2014	08:10	4230.7767	1422.8233	65	Microbiology	
7	SW104-CORER 3	Short core	14/10/2014	08:36	4230.7863	1422.8292	65	x-ray, magnetic susceptibility	47 cm long (whole)
31	GRAB	Surface	14/10/2014	09:01	4231.6052	1424.4080	77	OC, OM, grain size, Hg, porosity	
32	GRAB	Surface	14/10/2014	10:51	4235.0848	1429.6133	105	OC, OM, grain size, Hg, porosity	
33	GRAB	Surface	14/10/2014	11:50	4240.0520	1437.0277	150	OC, OM, grain size, Hg, porosity	
56	GRAB	Surface	14/10/2014	13:02	4245.9748	1446.3410	199	OC, OM, grain size, Hg, porosity	
57	GRAB	Surface	14/10/2014	13:58	4251.6400	1444.8425	268	OC, OM, grain size, Hg, porosity	
57	SW104-CORER 1	Short core	14/10/2014	14:15	4251.6658	1444.9518	268	OC, OM, grain size, Hg, porosity	20 cm long (sliced @ 2 cm)
57	SW104-CORER 2	Short core	14/10/2014	15:14	4251.5463	1444.9543	267	x-ray, magnetic susceptibility	63 cm long (whole)
34	GRAB	Surface	15/10/2014	04:47	4159.7572	1514.7940	31	OC, OM, grain size, Hg, porosity	
35	GRAB	Surface	15/10/2014	05:14	4201.4463	1515.0250	49	OC, OM, grain size, Hg, porosity	

Appendix B

Station	Instrumentation	Subsampling	Date	Time (UTC)	Latitude GGMM.xxx	Longitude GGMM.xxx	Corr. water depth (m)	Planned analysis*	Comments
36	GRAB	Surface	15/10/2014	05:46	4203.0478	1515.5365	69	OC, OM, grain size, Hg, porosity	
37	GRAB	Surface	15/10/2014	06:19	4204.0500	1516.5500	79	OC, OM, grain size, Hg, porosity	
38	GRAB	Surface	15/10/2014	06:54	4207.6243	1516.3835	92	OC, OM, grain size, Hg, porosity	
55	GRAB	Surface	15/10/2014	07:41	4213.1395	1518.2920	112	OC, OM, grain size, Hg, porosity	
5	GRAB	Surface + short core	15/10/2014	11:55	4202.0425	1608.1143	75	Microbiology + Gargano flood	19 cm long
6	GRAB	Surface + short core	15/10/2014	12:26	4159.2908	1608.9427	35	Microbiology + Gargano flood	18.5 cm long
6	SW104-CORER	Short core	15/10/2014	12:44	4159.3590	1609.9937	36	Microbiology	
75	GRAB	Surface + short core	15/10/2014	13:43	4157.0598	1617.5815	57	Microbiology + Gargano flood	18.5 cm long
74	SW104-CORER	Short core	15/10/2014	14:36	4155.3058	1615.2290	33	Gargano flood	61 cm long
73	SW104-CORER	Short core	15/10/2014	15:07	4154.1627	1613.0837	20	Gargano flood	59 cm long
39	GRAB	Short core	15/10/2014	15:59	4148.3242	1616.3265	20	Gargano flood	20 cm long
40	GRAB	Short core	15/10/2014	16:29	4149.1403	1619.3827	34	Gargano flood	20.5 cm long

Station	Instrumentation	Subsampling	Date	Time (UTC)	Latitude GGMM.xxx	Longitude GGMM.xxx	Corr. water depth (m)	Planned analysis*	Comments
41	GRAB	Short core	15/10/2014	16:50	4149.3230	1621.1147	47	Gargano flood	21 cm long
42	GRAB	Short core	15/10/2014	17:51	4150.3083	1623.5863	66	Gargano flood	21 cm long
43	GRAB	Surface	16/10/2014	05:49	4203.8727	1641.4057	201	OC, OM, grain size, Hg, porosity	
44	GRAB	*FAILED*	16/10/2014	*FAILED*	*FAILED*	FAILED			
45	GRAB	*FAILED*	16/10/2014	*FAILED*	*FAILED*	FAILED			
3	OCEANIC BOX CORER	Surface + 2 short cores	16/10/2014	13:56	4157.6053	1708.0223	801	OC, OM, grain size, Hg, porosity	22 cm long (sliced @ 1 cm down to 10 cm; 12-14; 16-18; 20-22 cm) 30 cm long (whole)
11	OCEANIC BOX CORER	Surface	16/10/2014	15:31	4154.5395	1658.3788	516	OC, OM, grain size, Hg, porosity	
2	OCEANIC BOX CORER	Surface + 2 short cores	17/10/2014	06:22	4138.0303	1741.6487	1039	OC, OM, grain size, Hg, porosity/ xray, magnetic susceptibility	20 cm long (sliced @ 1 cm down to 10 cm; 2 cm between 10-20 cm) 36.5 cm long (whole)

Station	Instrumentation	Subsampling	Date	Time (UTC)	Latitude GGMM.xxx	Longitude GGMM.xxx	Corr. water depth (m)	Planned analysis*	Comments
54-1200	OCEANIC BOX CORER	Surface	17/10/2014	08:26	4137.8432	1737.8938	1180	OC, OM, grain size, Hg, porosity	
12	OCEANIC BOX CORER	Surface	17/10/2014	10:45	4144.5452	1735.8562	1064	OC, OM, grain size, Hg, porosity	
52	OCEANIC BOX CORER	Surface	17/10/2014	12:46	4146.0743	1717.6820	990	OC, OM, grain size, Hg, porosity	
53-720	OCEANIC BOX CORER	Surface	17/10/2014	14:29	4148.5275	1701.9908	718	OC, OM, grain size, Hg, porosity	
47	OCEANIC BOX CORER	Surface	17/10/2014	15:49	4143.1133	1707.0035	819	OC, OM, grain size, Hg, porosity	
70	GRAB	Surface	18/10/2014	05:38	4109.7377	1652.6168	55	Microbiology, Hg	
71	SW104-CORER	Short core	18/10/2014	06:30	4110.6878	1653.6983	81	Microbiology, Hg	50 cm long (sliced @ 2 cm)
72	GRAB	Surface	18/10/2014	07:12	4113.1440	1656.4485	109	Microbiology, Hg	
49	OCEANIC BOX CORER	Surface	18/10/2014	09:04	4118.1953	1712.2093	600	OC, OM, grain size, Hg, porosity	
48-609	OCEANIC BOX CORER	Surface	18/10/2014	10:00	4120.2888	1711.9213	608	OC, OM, grain size, Hg, porosity	
69	OCEANIC BOX CORER	Surface	18/10/2014	11:33	4125.6225	1713.9385	650	OC, OM, grain size, Hg, porosity	
1	OCEANIC BOX CORER	Surface + 2 short cores	18/10/2014	12:53	4121.7060	1719.0145	709	OC, OM, grain size, Hg, porosity/ xray, magnetic susceptibility	30 cm long (sliced @ 1 cm down to 10 cm; 2 cm between

Station	Instrumentation	Subsampling	Date	Time (UTC)	Latitude GGMM.xxx	Longitude GGMM.xxx	Corr. water depth (m)	Planned analysis*	Comments
									10-30 cm) 44 cm long (whole)
50-858	OCEANIC BOX CORER	Surface	18/10/2014	14:59	4113.0752	1735.0667	858	OC, OM, grain size, Hg, porosity	
68	OCEANIC BOX CORER	Surface	19/10/2014	08:26	3959.5095	1857.9980	888	OC, OM, grain size, Hg, porosity	
67	OCEANIC BOX CORER	Surface	19/10/2014	09:46	3959.4972	1852.0272	815	OC, OM, grain size, Hg, porosity	
13	OCEANIC BOX CORER	Surface + 2 short cores	19/10/2014	10:56	3959.5335	1847.7452	599	OC, OM, grain size, Hg, porosity/ xray, magnetic susceptibility	30 cm long (0-2 cm; sliced @ 1 cm between 2-20 cm; 2 cm between 20-30 cm); XX cm long (whole)
A5	OCEANIC BOX CORER	Surface	19/10/2014	12:54	3959.9805	1834.1745	102	OC, OM, grain size, Hg, porosity	
14	OCEANIC BOX CORER	Surface	19/10/2014	14:21	4007.7348	1836.6235	82	OC, OM, grain size, Hg, porosity	
46	OCEANIC BOX CORER	Surface	21/10/2014	11:03	4152.7763	1700.4177	573	OC, OM, grain size, Hg, porosity	
44	OCEANIC BOX CORER	Surface	21/10/2014	12:56	4206.4292	1657.0410	361	OC, OM, grain size, Hg, porosity	

APPENDIX C: Extraction of polychlorinated biphenyls (PCBs) in spiked marine sediments using accelerated-solvent extraction (ASE) and Soxhlet extraction

Extraction of PCBs in spiked marine sediments using accelerated-solvent extraction (ASE) and Soxhlet extraction

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Riassunto
In questo lavoro, l'estrazione convenzionale con Soxhlet e quella accellerata con solvente (ASE) sono state confrontate per valutare il recupero dei bifenili clorurati (PCBs) in sedimenti marini. I campioni di sedimento sono stati sottoposti ad entrambe le tecniche di estrazione, previa aggiunta di una quantità nota di standard di PCBs. La quantificazione dei PCBs è stata effettuata mediante gas cromatografia con rivelatore a cattura di elettroni (GC-ECD). Il recupero dei *congeneri dei PCB è risultato leggermente più elevato con il Soxhlet rispetto all'ASE (96 ± 14 e 76 ± 29%, rispettivamente, ed entrambe le tecniche hanno mostrato recuperi inferiori per i congeneri dei PCB a basso grado di clorurazione rispetto a quelli altamente clorurati. L'estrazione con ASE può essere considerata metodo estrattivo alternativo ed efficiente per l'analisi dei congeneri dei PCB in sedimenti marini.*

Summary
In this work, Soxhlet extraction and accelerated-solvent extraction (ASE) were compared for recovering of polychlorinated biphenyls (PCBs) in spiked marine sediment samples. Uncontaminated sediment samples were spiked with PCB standard and were analysed by both procedures. The determination of PCBs was performed by gas chromatography (GC) with electron capture detection (ECD). The mean recovery for PCB congeners was slightly higher for Soxhlet than for ASE extraction (96 ± 14 and 76 ± 29%, respectively), and both techniques presented lower recoveries of low-chlorinated PCBs in comparison with the recoveries of high-chlorinated PCBs. Although the ASE recoveries were modestly lower than Soxhlet, the results were satisfactory. On this way, the ASE extraction could be considered a useful and efficient method for PCB congeners analyses in marine sediments.

1. Introduction

The quantification of PCBs in environmental samples is performed through several steps, such as sampling, extraction, purification and instrumental analysis. Within these steps, the extraction is of major importance since it is responsible for the removal of the compounds from the matrix of interest to the solvent [1][2].
The Soxhlet method has been widely used for the extraction of organic compounds, including PCBs, from sediment samples because it usually provides acceptable recoveries and reliable

data. Despite the efficiency of the Soxhlet extraction, this technique presents some problems as the usage of large volumes of solvents, which in some cases are toxic, and longer extraction time compared to other methods [3]. For these reasons, alternative extraction procedures for PCBs and other organic compounds is a common interest.
Techniques such as accelerated solvent extraction (ASE), microwave-assisted extraction (MAE) and ultrasonic agitation/sonication (US) are alternatives with recognized advantages, including less use of organic solvents, elimination of clean-up step and less time consumption [2]. Nevertheless, the efficiency of alternative methods has been considered generally lower than Soxhlet extraction. Some studies considered the US as poorly efficient for some organic compounds in the tested environmental matrices [3] [4].
In this study, Soxhlet extraction and ASE were compared for recovering of polychlorinated biphenyls (PCBs) in spiked marine sediment samples from the Adriatic Sea.

2. Report

2.1 Material and methods

2.1.1 Sample preparation
The sub-surface sediment samples used in this work were collected with a box corer close to the region of Gargano Peninsula, in the Southern Adriatic Sea. After sampling, the sediments were kept in the freezer and subsequently air-dried and stored in a glass bottle until laboratory analysis.
Both methods, ASE and Soxhlet extraction, were tested in triplicates. For each replicate, approximately 10 g of sediments were weighed into a glass mortar and ground into a homogeneous free-flowing powder. The sediment samples were spiked at 10 ng/mL with a PCB standard containing the following congeners: PCB 8, PCB 18, PCB 28, PCB 44, PCB 52, PCB 66, PCB 77, PCB 81, PCB 101, PCB 105, PCB 114, PCB 118, PCB 123, PCB 126, PCB 128, PCB 138, PCB 153, PCB 156, PCB 157, PCB 167, PCB 169, PCB 170, PCB 180, PCB 187, PCB 189, PCB 195, PCB 206 and PCB 209 (C-WNN, AccuStandard, USA). TCMX (tetrachloro-m-xylene, AccuStandard, USA) was also added to the samples as surrogate standard. A procedural blank was performed for each method using 10 g of sodium suphate anhydride (Sohxlet) and 10 g of diatomaceous earth (ASE) and analysed in the same way as samples.

2.1.2 Soxhlet extraction
The samples were placed in cellulose thimbles with 2 g of sodium sulfate and extracted during 16 h using 100 mL of a mixture of acetone and n-hexane (20:80) in a Soxhlet apparatus. The heating mantle temperature was set to allow approximately four solvent cycles per hour.

2.1.3 ASE
The samples were placed in stainless-steel cells with 2 g of diatomaceous earth and extracted with approximately 50 mL of a mixture of acetone and n-hexane (50:50). The extraction was carried out using the ASE 350 system (Dionex, USA). The extraction procedure consisted of three cycles, where the samples were pre-heated for 5 minutes and extracted for 5 minutes at 100°C at a pressure of 1500 psi in each cycle. After the three cycles, the cell was rinsed with the extraction solvent (about 60% of cell volume) and purged with N_2 stream at 150 psi for 180 s to remove the residual solvent on the cell [3].

2.1.4 Clean-up
The extracts from both methods were concentrated up to 2 mL and the purification was carried through the same procedure. The extracts were purified using column chromatography

with 1 cm of deactivated silica, 5 g of acidic silica (30% H_2SO_4) and 1 cm of sodium sulfate. Activated copper was added to remove sulphur. The samples were eluted with 60 mL of a mixture of dichloromethane (DCM) and n-hexane (40:60). The extracts were concentrated up to a final volume of 1 mL under a gentle gas stream of purified nitrogen, and PCB 198 (AccuStandard, USA) was added as internal standard.

2.1.5 Instrumental analysis

The determination of PCBs was performed by gas chromatography (Varian CP 3800) with electron capture detection (ECD). The GC-ECD column was an MDN-5S (Supelco) (length: 30 m, ID: 0.25 mm, film thickness: 0.25 µm). The oven temperature started with 100 °C for 2 minutes, increasing at 15°C by minute until 160°C and increasing 5°C until 270°C with a final hold time of 10 minutes.

Instrument calibration was made with a 5-point calibration curve prepared with PCB standard C-WNN (AccuStandard, USA) at the following concentrations: 1, 2, 5, 10 and 20 ng/mL. The analytes were identified by comparison of the retention times of the peaks detected in each replicate with the peaks obtained on the calibration curves. The quantification of the PCBs was based on the area obtained for each analyte in the samples, according to the mass/area ratio obtained for the internal standard and on the response factor obtained from the calibration curve

2.2 Results and discussion

The analysis of the blanks showed no external interference for the tested methodologies. The mean surrogate recoveries, based on the relationship with the internal standard (PCB 198) added before the GC-ECD analyses, were 90 ± 11 % for ASE and 92 ± 5 % for the Soxhlet extraction.

The ASE recoveries for PCB congeners ranged from 21% for PCB 18 to 124% for PCB 189. For the Soxhlet extraction, the lowest and highest recoveries were 72% for PCB 101 and 120% PCB 206, respectively. The mean recovery for total PCBs (∑28 congeners) was slightly higher for Soxhlet extraction than for ASE (96 ± 14 and 76 ± 29%, respectively) (Table 1), which is in agreement with the results detected on previous works [2][3][4].

The relative standard deviation (RSD), or coefficient of variation, is a ratio between the value of the standard deviation and the value of the mean. This parameter reflects the precision of the method and showed a mean value of 7% for ASE and Soxhlet extractions, indicating satisfactory repeatability for both methods.

	ASE	Sohxlet
Maximum recovery	124	120
Minimum recovery	21	72
Mean recovery ± SD	76 ± 29	96 ± 14
RSD	7	7

SD: standard deviation; RSD: relative standard deviation.

Tab. 1 – *PCBs recoveries (%) for ASE and Soxhlet extraction.*

PCBs are often reported according to the degree of chlorination of the congeners. The degree of chlorination, as well as the molecular weight of PCBs, is related to the number of chlorine atoms in the molecules [5]. PCBs are made up of a biphenyl nucleus with 1 to 10 chlorine

atoms and the congeners with the same number of chlorine atoms are known as homologs [6]. The average recovery for each group of homologs is shown on Figure 1. The octa, nona and deca-CBs were grouped because only one congener for each group was analysed. The ASE recoveries ranged from 71 ± 3% (tetra-CB) to 97 ± 4% (penta-CB). The lowest recovery for the Soxhlet extraction was 85 ± 6% (tetra-CB) and the highest was 111 ± 3% (octa, nona and deca-CB).

The Soxhlet average recoveries are higher than ASE for all the groups except for the penta-chlorinated PCBs. Nevertheless, no significant differences were detected for tetra, penta and hepta-CB mean recoveries (Student t-test, $p > 0.05$). The recovery was significantly different for the hexa-CB and for the group formed by the octa, nona and deca-CB (Student t-test, $p < 0.05$).

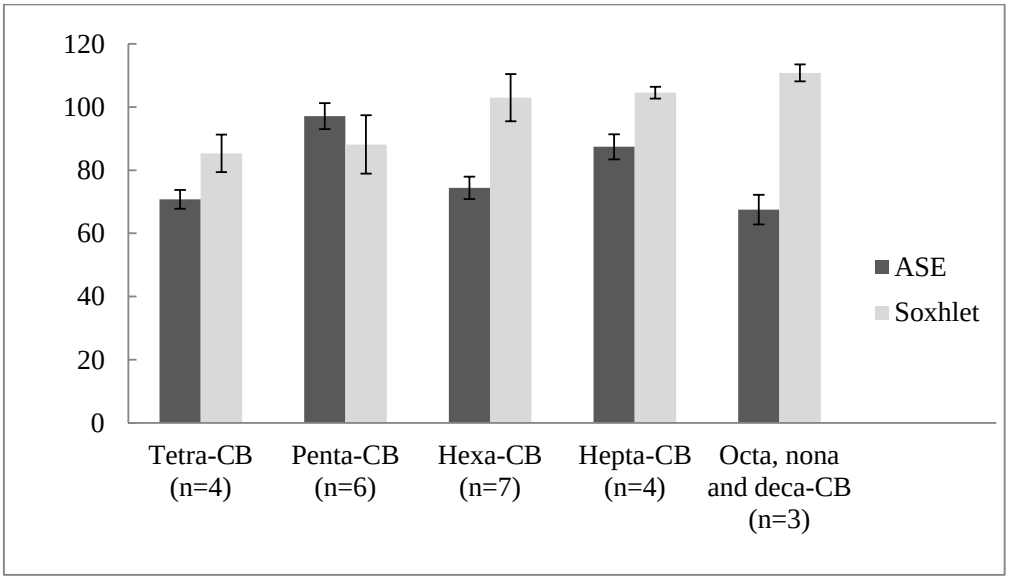

Fig. 1 – *Average PCBs recovery (%) according to the degree of chlorination. Error bars indicate the standard deviation; n is the number of congeners in the homologue group.*

The degree of chlorination determines the molecular weight of PCBs, being also responsible for the properties of each PCB congener [5][6]. Therefore, the congeners that present 1 to 4 chlorine atoms in their structure are known as low-chlorinated PCBs. These congeners are less toxic, more soluble in water and more volatile. On the other hand, PCBs presenting 5 to 9 chlorine atoms are considered high-chlorinated PCBs, which are more toxic and less susceptible to transport and degradation [6][7][8].

Considering these properties, the mean recoveries of low-chlorinated PCBs were lower for both extraction methods (57 ± 6% for ASE and 84 ± 6% for Soxhlet) in comparison with the mean recoveries of the high-chlorinated congeners (83 ± 4% for ASE and 100 ± 6% for Soxhlet).

Four low-chlorinated congeners (PCB 18, PCB 28, PCB 52 and PCB 44) showed the lowest recoveries with the ASE procedure, with a mean value of 25 ± 3%, which is far below the recoveries obtained for other congeners. This problem was not detected for the Soxhlet extraction. Considering that all the analytical steps were the same for both methods, this fact suggests a loss of these congeners during the ASE extraction.

3. Conclusions

Overall, the ASE recoveries were slightly lower in comparison with Soxhlet. However, they fall within the acceptance range commonly adopted in scientific literature (between 40 to 130%) for the majority of the congeners analysed. Furthermore, the relative standard deviation, or coefficient of variation, showed that both methods present excellent repeatability (RSD < 10%).

The mean recovery of low-chlorinated PCBs was lower than the mean recovery of high-chlorinated PCBs. This difference was especially important for ASE extraction, suggesting a loss of the more volatile congeners during this type of extraction, which could be overcome with further optimization on time, pressure, and temperature conditions.

Considering the advantages associated to the ASE technique, including a substantial minimization on the usage of organic solvents (reduction of 50% on the solvent volume) and extraction duration (from 4 to16 hours for Soxhlet versus 30 minutes for ASE), ASE extraction could be considered a faster and efficient alternative method for PCB congeners analyses in marine sediments.

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